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THE BIOCHEMISTRY OF THE AMERICAN PITCHER PLANTS

HEPBURN, ST. JOHN, AND JONES

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BIOCHEMICAL STUDIES OF THE NORTH AMERICAN SARRACENIACEAE

By

JOSEPH SAMUEL HEPBURN, A.M., B.S. IN CHEM., M.S., PH.D.
FRANK MORTON JONES, F.E.S.
and
ELISABETH QUINTARD ST. JOHN, M.D.

CONTRIBUTIONS FROM THE CONSTANTINE HERING RESEARCH LABORATORY, HAHNEMANN MEDICAL COLLEGE OF PHILADELPHIA

75882

INTRODUCTION

THE observations and experiments forming the basis of the following papers on the North American *Sarraceniaceæ* had their beginning in 1892 when *Sarracenia flava*, *S. rubra*, and *S. purpurea* came under observation in Richmond County, North Carolina. In succeeding years all the species have been under frequent observation in their native habitats, and have been made the subject of several papers on insect-plant relations by one of us (Jones).

Field and laboratory experiments on the biochemistry of the *Sarraceniaceæ* were commenced in 1917, and have been continued until the present time. These researches form part of a general study of insectivorous plants, work on *Nepenthes* having been begun by Hepburn and St. John in 1914. *Dionaea* has been made the subject of a recent paper.

Observations and field experiments have been made or material for laboratory examination collected chiefly near the following localities for each species:

Darlingtonia californica.—Keddie, Plumas Co., and Mt. Eddy (the type locality), Siskiyou Co., Cal.

Sarracenia minor.—Summerville, Berkeley Co., S. C.; Jacksonville, Duval Co., Fla.

Sarracenia Sledgei.—Mobile, Theodore, and Bayou La Batre, Mobile Co., Ala.; Biloxi, and Wiggins, Harrison Co., Miss.

Sarracenia flava.—Southern Pines, Moore Co., Hamlet, Richmond Co., and Wilmington, New Hanover Co., N. C.; Summerville, Berkeley Co., S. C.; De Funiak Springs, Walton Co., Fla.; Bay Minette, Baldwin Co., Ala.

Sarracenia Drummondii.—De Funiak Springs, and Freeport, Walton Co., Fla.; Bayou La Batre, and Theodore, Mobile Co., and Bay Minette, Baldwin Co., Ala.

Sarracenia rubra.—Southern Pines, Moore Co., and Hamlet, Richmond Co., N. C.; De Funiak Springs, Walton Co., Fla.

Sarracenia purpurea.—Tolland, Tolland Co., Conn.; Whitings, and Toms River, Ocean Co., N. J.; Pocono Pines, Monroe Co., Pa.; and many other localities from Maine to Mississippi.

Sarracenia psittacina.—De Funiak Springs, Walton Co., Florida; Bayou La Batre, Mobile Co., and Bay Minette, Baldwin Co., Ala.; and Ocean Springs, Jackson Co., Biloxi, and Wiggins, Harrison Co., Miss.

Samples of pitcher liquor and aqueous solutions of the nectar, which were collected in the field for laboratory examination at Philadelphia, were preserved by addition of 0.2 percent of trikresol. The collection of material for bacteriological study and for chemical examination of the plant tissues is described in detail in the papers on these respective subjects.

In this series of papers the authors have followed Macfarlane[95] in the nomenclature of the *Sarraceniaceæ*. They are indebted to Dr. Macfarlane for many helpful suggestions in the course of these researches.

The authors record their indebtedness to the Franklin Institute of the State of Pennsylvania for permission to use portions of a paper on absorption of nutrients and allied phenomena in the pitchers of the *Sarraceniaceæ*, originally published in the *Journal of the Franklin Institute*, February, 1920. The Institute has also permitted the reprinting of several illustrations from its *Journal*.

The American Museum of Natural History has granted permission to reprint three illustrations from its journal, *Natural History*.

WORK OF PREVIOUS INVESTIGATORS ON THE BIOCHEMISTRY OF THE SARRACENIACEÆ

By JOSEPH SAMUEL HEPBURN, A.M., B.S. in Chem., M.S., Ph.D.

DIGESTION AND ABSORPTION

MACBRIDE[1] studied *Sarracenia adunca* (*S. minor*, *S. variolaris*) and *S. flava* in their native habitat. His observations were made chiefly during 1810 and 1811 and were communicated to the Linnean Society of London in 1815. He noted the masses of captured insects within the pitchers and wrote: "What purposes beneficial to the growth of these plants may be effected by the putrid masses of insects, I have never ascertained."

Hooker[2,3] found "a slight acid secretion" in young pitchers of *Darlingtonia californica*. He states that the pitchers of both *Sarracenia variolaris* (*S. minor*) and *S. flava* secrete a fluid. However, the pitchers of *S. variolaris* examined by him contained no secretion. He also examined both half-grown and full-grown pitchers from cultivated plants of *S. flava;* they contained no fluid except what may have been accidentally introduced. According to Hooker, secretion of "water" by the pitchers of *S. purpurea* had never been observed, and he suggested "the possibility of this plant either having no proper secretion of its own, or only giving it out after the pitcher has been filled with rain water." He considered it quite likely that the "digestive functions" in *Sarracenia* pitchers may be of short duration. Digestion experiments were not made with the *Sarraceniaceæ*.

Mellichamp[4] made observations on *Sarracenia variolaris* (*S. minor*) growing in its native habitat in the vicinity of Bluffton, South Carolina. Unopened pitchers usually contained from 3 to 5 drops of liquor, occasionally as many as 10 drops, rarely 15 drops. The liquor was bland and somewhat mucilaginous to the taste; it left an astringent taste in the mouth. Perfect, open pitchers usually contained from 10 to 15 drops of liquor, very rarely a half-drachm. Mellichamp adds: "*I have, however, since found the fluid much increased in quantity*, very frequently a drachm, and *sometimes as much as two*

drachms." He states that the secretion of pitcher liquor "appears to continue during the whole period of the entrapment, which I suppose does not last over two or three weeks at most."

"During this active period the 'water' is attracted to the top of the mass and permeates and percolates through every portion of it so that eventually all the soft parts of the insects are thoroughly dissolved. After these animal juices have been partially or entirely absorbed by the plant, or by the larva of the Sarcophaga fly which continues to grow and fatten upon this rich diet; the remaining portion, commences gradually to dry, until only the backs and legs and shells of the various insects remain."

The digestive action of the pitcher liquor was tested on venison, apparently *in vitro*, without a bactericide. Bits of fresh venison were immersed in the pitcher liquor and in a corresponding amount of pure water. After the lapse of 15 hours, the venison in the pitcher liquor was more changed, softened, and broken up, and far more offensive to the nostrils (being offensive to a disgusting degree) than was the venison in the water. Mellichamp concluded that this experiment perhaps showed that the liquor hastened the decomposition of the insects and their conversion into "liquid manure."

Edwards[5] considered that the pitcher liquor of *Darlingtonia californica* did not possess true digestive power, but was able to "cause decomposition" of the prey. He stated: "I do not attempt to speak authoritatively upon the subject, but I am inclined to think that no process similar to digestion goes on within the plant, but that the fluid mass derived from the decay of the imprisoned insects descends through the tube into the earth, and is taken up by absorption, through the roots, thus acting as a kind of liquid manure. It is true that in the dead leaves the hard integuments of insects, such as the elytra of beetles, and the bodies of wasps and hornets are to be found undecayed, but this may be because the liquid secreted by the plant is not powerful enough to cause decomposition of these parts before the plant itself decays." He commented on the "most disgusting" smell of the pitcher contents, and recorded that "after handling a number of specimens of the tubes, it is necessary to use some disinfectant like ammonia or chloride to remove the disagreeable odor."

The statements of Canby[6] concerning *Darlingtonia californica* were based in part on the observations of his correspondent, Lemmon. Canby mentions the secretion of a pitcher liquor by *Darlingtonia*. "Several inches

of the bottom of the tube are filled with a clear fluid (secreted by the leaves it must be). . . . Mr. Lemmon has kindly sent me an ounce phial completely filled with the fluid 'from two petioles' . . . It is scarcely necessary to say, that as it is certain no water can get into the tube by any ordinary means, and as the fluid is always present in healthy leaves, it must be secreted by the plant as Mr. Lemmon says."

Canby also mentions the offensive odor of a patch of *Darlingtonia* plants whose pitchers contained prey. "Mr. Lemmon further says 'I came upon a patch once in September and smelled it from afar so offensive was it. A portion of the leaves filled with insects to the depth of four to six inches, had fallen down apparently from the weight of the fluid and insects.'"

The response of *Darlingtonia californica* to food stimulation was discovered by Mrs. R. M. L. Austin who studied these plants in their native habitat, Butterfly Valley, Plumas County, California. Mary E. Pulsifer Ames[7] mentions Mrs. Austin's researches, and publishes the following extract from a letter received by herself from Mrs. Austin. "In July, 1875, I fed a great many of the leaves, some with fresh raw mutton and others with that which was boiled. The liquid, in the course of a week, would fill the tubes and flow out of the orifice." Asa Gray[22] states that Mrs. Austin, studying *Darlingtonia californica*, found that "the watery liquid in the pitcher, which must be wholly a secretion, is much increased in quantity after the capture of insects."

Batalin[8] described certain changes in the cell-wall of the pitcher-lining of *Darlingtonia californica* and of several species of *Sarracenia* after prey had been captured. He concluded that these changes facilitated the absorption, by both genera, of the products derived from the captured insects.

Pitchers of green-house plants of *Sarracenia flava* never contained any liquor, although their inner surface was frequently moist. The outer surface of the pitcher always was green. The inner surface of the lower part of the pitcher was green when insects had not yet been captured, and became a brownish yellow at those places where captured insects adhered to the wall. This change affected only the interior surface of the pitcher, and occurred only in its lower or detentive zone. Sections of the inner surface of this zone were cut. When the inner surface was green and free from adhering insects, the cavity surface of the epidermal cells was quite smooth, homogeneous, and free from markings of any kind. When insects were adhering to the epidermal cells, the cavity surface of each cell contained one or two paler areas surrounded

by a more or less broad, intense yellowish-brown margin which lay at a higher level. The epidermal cells are provided with a cuticula which forms the outermost layer of the cell membrane. In cells, to which insects had adhered, the cuticula had become detached from the paler colored areas; the margin was the remaining portion of the cuticula. Cells, which adjoined the altered cells but were free from insects, had not lost any of their cuticula and were free from paler areas.

Some sections revealed the manner in which the cuticula was detached. Bubbles appeared, as if some substance were secreted, between the cellulose membrane and the cuticula. The bubbles were of a brighter yellow than the cuticula. The secreted substance, the nature of which is unknown, gradually collected in greater amount, produced a further separation of the cellulose membrane from the cuticula, rendered the latter yellow and viscous or gelatinous, and finally caused detaching of the cuticula. At times not only the cuticula, but possibly the entire cuticular layer was cast off from the paler areas. The secreted substance owed its origin to a stimulus exerted on the epidermal cell by the adhering insect. Occasionally the cuticula was detached from as many as ten areas on a single cell; these areas were then surrounded by a yellow network of the remaining cuticula. The detentive hairs spring from cells which are smaller than those participating in these changes.

In *Sarracenia purpurea*, the change in the cuticula was almost the same as in *S. flava*. In the lower portion of the wide-open pitcher, each of the altered cells lost the cuticula from an oval area or from two areas. In the narrower portion of the pitcher, near its bottom, the cuticula might be cast off from as many as ten or twelve areas on a single cell and, at times, might become detached from a number of adjacent cells as a thin unbroken membrane which showed distinctly the contour of the individual cells.

In old pitchers of *Sarracenia variolaris* (*S. minor*) which had not captured insects, the cuticula was not homogenous in the lower or detentive zone, but was extremely thin or entirely lacking on certain regions, especially on the clefts which occurred at the outer edge of the cuticula where it met the inner border of the projection of the side wall of the cell. These clefts consisted of two or three rows of extremely small points or irregular, four-cornered, rounded areas, or of a single row of larger areas; occasionally these larger areas united into two or three still larger areas, between which were smaller areas or points. Other points and areas of this type occurred on the surface of the cuticula.

When the cuticula was cast off, detachment usually took place along these clefts so that only narrow strips of cuticula remained as scollops. At times, a cell would lose the cuticula from two or three isolated areas instead of a single large area. The remnants of the cuticula were usually yellow, and apparently gelatinized; the cuticula-free surface of the cell and its side walls were almost colorless.

Only old pitchers of *Darlingtonia californica* were available for study. In the detentive zone, the epidermis resembled that of *Sarracenia variolaris*. The areas, on which the cuticula was extremely thin or entirely lacking, were similar to those of *S. variolaris* with respect to both shape and distribution; occasionally they were so strewn over the entire surface of the cell that it resembled a sieve-plate. The cuticula was thus modified only in those parts of the pitcher cavity which contain long, stiff hairs. These modified areas were almost entirely lacking in the upper region with its short thin hairs. Clefts of large surface area predominated in the middle portion of the pitcher. In the lower, narrow portion, the modified areas were points, scattered over the cuticula in such large numbers that its surface was granular. Detachment of the cuticula could occur in all parts of the cavity of the pitcher; it was cast off from the entire surface of the cell along the line of the modified areas; only a remnant of cuticula was left between adjoining cells.

Zinc chloride plus iodine was of service as a stain in study of the sections.

Batalin drew the following conclusions from his research: "The physiological significance of the phenomena here described is quite clear. They can be considered as processes which facilitate for the plant the absorption of dissolved substances from without. It has been known for a long time that the cuticula is that part of the epidermis membrane which offers the greatest resistance to the penetration of substances into the cell. Therefore one can look upon its detachment as an adaptation for facilitating this entrance of substances into the plant. Accordingly in these two genera we have an interesting example of adaptation of the plants for particular purposes. . . . In *Sarracenia* and *Darlingtonia*, in consequence of the almost complete lack of glands in the pitchers, their function is here taken over by the entire inner surface; the epidermis is changed for this purpose in such a way that the absorption of solutions is rendered possible. From the described process, the manner and way in which the cuticula is cast off, it is seen that a substance is secreted between it and the cellulose membrane. How Nature has provided

this secretion is unknown to me; it is not improbable that it contains the solvent (Lösungsmittel) for the digestion of the proteins."

"These pitcher plants must be grouped with those which satisfy their nitrogen requirement through the captured insects. . . . They take up the nitrogen which they require directly from the insects . . . and not indirectly by manuring the soil on the surface of which the dead leaves plus the captured insects decay."

Schimper[9] studied *Sarracenia purpurea* growing wild on the Massachusetts coast. When the plants grew in the open, the pitchers usually contained water, i. e., liquor. Observations made on plants under cultivation led to the conclusion that a very small portion of this liquor is secreted by the pitcher itself; the major portion owes its origin to the rain. Secretion of liquor occurs in young pitchers, indeed long before opening. Acid-reacting droplets occur on the lower hairy region as well as on the middle region of smooth epidermis, and collect at the bottom of the pitcher.

Digestion experiments were made; small pieces of meat were introduced into several pitchers; other pitchers were permitted to catch insects. A control experiment was made at the same time by introducing small pieces of meat into water contained in a glass vessel. The meat dissolved very slowly in the liquor within the pitchers; in fact, the rate of solution did not exceed that in the control experiment; hence it was concluded that the pitcher liquor did not contain pepsin. Moreover, the presence of bacteria was definitely shown. Introduction of nitrogenous compounds did not increase the acidity of the pitcher liquor.

Schimper stated: "Innumerable worms were present in all the leaves studied, these possibly participate in the transformation of the animal bodies into soluble compounds." This may be construed to mean that the larvæ of the insect associates possibly play a part in the digestion of the prey.

The decomposition products of the prey were absorbed. This was definitely shown by certain changes which occurred in the epidermis cells in the bottom portion of the pitcher, and, to a lesser degree, in the cells of the adjacent subepidermal layer. As already stated, some pitchers were nourished by captured insects or by introduced meat. Other pitchers were, in a sense, starved by plugging the mouth with tissue paper as soon as the lid had opened. A striking difference was observed between the nourished and the starved pitchers. The cell sap of the cells in question is rich in tannin; it formed one,

two, or more very strongly refractive, glittering drops in the nourished pitchers; in starved pitchers it formed, at the most, a single drop which possessed these properties to a very slight degree and occupied a much larger space. The drops were not suspended in the cell sap, but represented the entire cell sap. Food caused a marked removal of the chlorophyll granules from the cell wall and their occurrence about the vacuoles or drops. The substances of animal origin in solution in the pitcher liquor acted as a stimulus; the protoplasm acquired greater power of imbibition, and withdrew water from the cell sap. The colloidal tannin was unable to pass through the protoplasm by osmosis and remained in the cell sap, which thus became more concentrated and acquired a higher refractive power, while the protoplasm became less refractive. These phenomena were not produced exclusively by nitrogenous compounds, but were obtained when either a dilute solution of sodium chloride, or sea water, or borax was used as a stimulus. Pure water was without stimulating action.

The "nourished" appearance was true of the cells at the base of the hairs even before the pitcher lid had opened. It was frequently noted at the border of a section in cells which had either been cut or rested directly on cut cells, and was then due to the disorganization of the cell contents. Therefore only somewhat thick sections were of value.

Schimper concluded that absorption certainly occurs through the entire interior surface of the hairy bottom portion of the pitcher. The thick-walled hairs have no function other than preventing the escape of the prey. If the pitchers contain very little liquor and many insects, the surface tissues become brown and decomposed. Pitchers of this type, which are extremely rare in plants growing in the open, apparently served Batalin in his studies. The property of the protoplasm to attain a greater degree of capacity for imbibition under the stimulus of certain substances appeared to be, in all likelihood, of direct significance for the nutrition of the plant. The very marked swelling would produce a widening of the micellar pores and a marked increase in the diosmotic properties of the protoplasm; as a result, the entrance into the tissues of the substances contained in the pitcher liquor would at least be facilitated, possibly be first made possible. Therefore the insect captures, in all probability, are actually of value to the plant.

Higley[10] made an extensive series of analyses of the pitcher liquor of *Sarracenia purpurea*, growing in the open, probably in Wisconsin.

His findings would indicate that the water content of the pitcher liquor is collected rain. "As a result of the examination of over 800 leaves I find that none contained any fluid before they had opened. . . . After opening there is no fluid till after the first rain except in a few cases when there has been a heavy dew."

One hundred analyses were made in order to ascertain the composition of the pitcher liquor before it contained any insects. Twenty-five of these analyses are published and may be summarized. The samples of pitcher liquor were collected from 2 to 20 days after the pitcher had opened to such an extent that rain could easily enter it, and from 1 to 18 days after rain had fallen. As to color, 13 samples were clear, 1 slightly yellow, 6 yellow, 3 tinged, and 2 dirty. The amount of solids and acids and bases, which were present, are reported in the table. The organic solids "consisted, to a great extent, of pollen, various other vegetable structures, Infusoria, algæ and the like." The reaction was "nearly neutral" in 4 samples, "slightly acid" in 11 samples, and "acid" in 10 samples.

CHEMICAL CONSTITUENTS OF THE PITCHER LIQUOR OF *SARRACENIA PURPUREA*, ACCORDING TO HIGLEY.

			Liquor prior to capture of insects.	*May samples.*	*June samples.*	*July samples.*	*August samples.*
Number of analyses published			25	10	10	10	10
Solids, parts per 1000	Organic..	Maximum	20	130	173	207	260
		Minimum	2	60	120	137	164
	Inorganic	Maximum	5	8	13	12	18
		Minimum	1	2	5	6	8
Number of samples containing	Ammonium*		25	10	10	10	10
	Sodium		12	9	10	10	10
	Potassium		24	10	10	10	10
	Magnesium		2	5	10	10	
	Calcium		19	8	10	10	10
	Aluminium		6	3	10	5	8
	Iron		4	3	7	7	10
	Silica		1		3	7	4
	Carbonates		25	10	10	10	10
	Chlorides		18	10	10	10	10
	Sulphates		9	3	10	5	8

On the last day of each month—May, June, July, and August—100 samples of pitcher liquor were collected, and analyzed; for each month, 10 typical analyses, including the extremes, were published. The data concerning solids and occurrence of acids and bases are summarized for each month in the table.

* A trace of nitric acid was probably present as ammonium nitrate in these samples.

The May samples were gathered from 1 to 23 days after rain had fallen. The reaction was acid in all 10 of the published analyses; the color of the liquor was "yellow" in 2, "dark" in 2, "dirty" in 3, and "wine" in 3 samples.

The June samples were collected from 1 to 7 days after rain had fallen; the reaction was acid in the entire 10 published analyses; 1 sample was "wine" color and 9 samples "dirty."

The July samples were obtained from 3 to 15 days after rain had fallen; the entire 10 published analyses were characterized by an acid reaction and a "dirty" color.

The August samples were collected from 5 to 20 days after rain had fallen; all 10 published analyses showed an acid reaction and a "dirty" color.

The pitchers, from which the liquor was procured for analysis, represented the growth of the season up to the date of collection. Each pitcher was cut open, and the contents were used only when they were of such consistency that they flowed easily.

The acidity of the pitcher liquor became greater each month, and was especially marked during July and August. "To just what acid, if any particular one, the reaction was due in the liquid of the earlier pitchers is not certain, but in the last two months both malic and citric acids appeared, the former in greater abundance."

The source of the malic acid is indicated: "It seems highly probable that the first lot of insects merely decay after maceration in the water first collected in the pitchers and that this mass acts not only as a stimulant to further decay but also to render the liquid more capable of absorbing certain organic principles from the leaf, such as Malic acid, which aid in the preparation of the abundant supply of food for absorption by the leaf. Thus the first mass might be called a digestive excitant."

Absorption occurred more rapidly during the last days of June and the months of July and August than earlier in the season; the cells then became filled with absorbed matter.

Ammonia was quite readily absorbed by the pitcher from the liquor. Several sets of experiments were made. In each set use was made of two pitchers of the same age and size, growing on the same plant, containing the same volume of liquor and approximately the same amount of insect remains; the pitchers chosen contained but a few insect remains. A quantitative determination of the "organic ammonia" was immediately made upon the fluid from

75882

one of the pitchers. The other pitcher was so turned and propped that rain could not enter, and was left thus for a week; the "organic ammonia" content of its pitcher liquor was then determined, and compared with the amount found in the first pitcher. "The fluid showed a decided decrease in each case, from the amount found in the one used in comparison. Though these analyses were perhaps quite far from sure in every detail, yet the average difference on comparison, viz.: sixty parts in one hundred would indicate quite rapid absorption, for such an amount could not possibly be removed in any other way." Therefore the pitchers absorbed nitrogenous compounds derived from "the decomposing remains of the insects."

Chemical examinations were made of the soils upon which the plants were growing. Not all the inorganic salts which were found in the pitcher liquor could be accounted for as from the soil.

Zipperer[11] found that the pitcher liquor of *Sarracenia purpurea* corroded starch grains after several days' action, and dissolved coagulated egg white in a short time. These results indicated that both a diastase and a peptonizing enzyme are secreted into the pitcher. Zipperer also made experiments with isopods of the genus *Oniscus*, either *Oniscus scaber* or *O. murarius*. The pitcher liquor first exerted a narcotic action on the prey; then death occurred as a result of suffocation; and finally, of the entire body, only a residue of chitin armor remained. He concluded that *Sarracenia purpurea* is a "finely developed insect trap, in the interior of which the insect is killed by a secretion, and assimilated by ferments."

Goebel[12] demonstrated that water and various solutions, introduced into *Sarracenia* pitchers, decreased markedly in volume in a few days. The water or solution was introduced into a pitcher of *Sarracenia illustrata* (a hybrid between *S. flava* and *S. purpurea*) until the level of the pitcher contents was 10 centimeters below the orifice; the latter was closed with a cork; and paraffin of low melting point was poured over the cork to prevent evaporation of the water. The level was marked by a strip of paper pasted on the exterior surface of the pitcher. The pitchers were examined 48 hours later with the following results:

One pitcher had received 20 cc. of 1 percent solution of formic acid and some swollen fibrin; the decrease in volume, due to absorption, was 6.8 cc.; the remaining solution still had an acid reaction; the fibrin appeared entirely unattacked.

Another pitcher had received 10 cc. of water, of which 2 cc. were absorbed.

A third pitcher had received 10 cc. of very dilute meat infusion exactly neutralized with sodium carbonate; the decrease in volume was 2.5 cc.; the meat infusion became alkaline in reaction and cloudy, and was full of bacteria.

A similar experiment was made with a pitcher of *Sarracenia Drummondii*. A 5 percent peptone solution, which had been introduced into the pitcher, underwent a decrease of somewhat more than 7 cc. in volume; the remaining solution occupied a volume greater than 2 cc., was markedly cloudy, and possessed a faint odor of putrefaction.

A young, green pitcher of *Sarracenia purpurea* was used in another experiment. A piece of meat the size of a barley grain and 10 cc. of water were introduced into the pitcher; and the orifice was closed airtight. Two days later 2.8 cc. of the water had been absorbed; the piece of meat was scarcely attacked, it was not foul, though thickly covered with bacteria.

Pitchers, each of which had received 5 cc. of meat juice and a small fragment of meat, were characterized 3 days later by a foul odor and the presence of ammonia. Therefore, whenever the pitchers contain a sufficient quantity of substances which are capable of putrefaction, that phenomenon makes its appearance.

The plants used in these experiments had previously been kept rather moist, in a cool north room. Under other conditions, the absorption would have been greater.

These conclusions are drawn. *Sarracenia* pitchers secrete neither a proteolytic enzyme nor a substance which prevents putrefaction. Digestive glands are not present. The inner surface of the pitcher, especially its lower portion, is able to absorb water and substances dissolved in the latter. Absorption occurs through the inner wall of the pitcher. The plant, as a rule, is but little sensitive to putrefaction products, provided they do not form in the pitchers in too great a quantity.

Goebel states that secretion of a pitcher liquor has never been observed in cultivated specimens of *Darlingtonia*. He suggests that the liquor of pitchers of plants growing in their native habitat may possibly exert an antiputrefactive action, provided too many insects are not captured. This genus was included in the absorption tests. A fragment of meat the size of a barley grain and 5 cc. of distilled water were introduced into each of three young pitchers of *Darlingtonia*. The orifice of each pitcher was then closed as tightly

as possible with cotton. Two days later the greater portion of the fluid had been absorbed; a portion had evaporated; the remaining fluid contained bacteria and moulds, but did not have a putrid odor. However, an odor of putrefaction was distinctly noticeable in the dead prey of other pitchers.

The presence of larvæ amid the prey in pitchers of *Sarracenia* and *Darlingtonia* was considered by Goebel as evidence that digestion of the prey does not occur in these two genera as rapidly as in *Nepenthes*. The identity of the compound, produced by digestion of the prey and absorbed by the pitcher wall, is not known. From experiments on *Nepenthes*, it is very probable that ammonia is absorbed. The specific cause of the digestion, whether autolysis of the prey or the activity of micro-organisms, is not stated.

Goebel concluded that *Darlingtonia* like *Sarracenia*, does not secrete a proteolytic enzyme in its pitchers. Secretion of a substance, which prevents putrefaction, does not occur in either genus, except, possibly, to a slight extent.

Lambert[13] made experiments on the secretion of pitcher liquor by *Sarracenia purpurea*, and on absorption from the pitchers of that species. His work apparently was done on the isle of Saint-Pierre and on the coast of Newfoundland.

The plants used in the secretion tests were kept under normal conditions. The liquor was withdrawn from the pitchers by means of a fine pipette; and every trace of moisture was removed with bibulous paper. The plant was placed beneath a shelter to exclude rain water, and the soil was watered amply. The interior of the pitchers remained perfectly dry.

Neither the introduction of several drops of ether nor the movements of a living insect produced any secretion of liquor by the pitchers. It had been hoped that these stimuli would cause secretion of fluid by the "stomach zone" into the dry pitcher cavity.

These results showed that the pitcher by itself does not secrete any fluid.

In the absorption tests, several drops of a solution of a crystalloid stain, such as methylene-blue of fuchsin, were added to the pitcher contents. Approximately 2 hours later the pitcher was cut open. In the pitcher wall of the bottom region, absorptive zone, or "stomach," the interior epidermis and one or two layers of subepidermal "digestive cells," which are characteristic of this zone, were stained blue or red according to the dye used. Staining of the pitcher wall occurred only in this zone. Colored colloids, *e. g.*, tincture of cochineal, were not absorbed and did not stain the tissues.

A highly concentrated solution of methylene-blue was left in living pitchers for an entire week. Only the epidermis and digestive cells were stained. The result was the same at the end of several hours as at the end of the week. After absorption, the stain was localized; and a limit was thereby placed on the total amount of absorption.

Lambert evidently considered that enzymic digestion of the prey, and absorption of the products occurred in the pitchers. "The albuminoid mass formed in the pitcher by the cadavers of the drowned insects will be attacked little by little by the liquid contained in the wall of the stomach, a liquid which passes through the wall as through a dialyzer. Under the action of this pepsin-like liquid, the albuminoids will be transformed into peptones and rendered assimilable, *i. e.*, will then be able to pass through the thin membrane of the epidermal cells in the stomach region and of the digestive cells which localize them."

The liquor is constantly agitated by living worms or annelids (larvæ?) in pitchers containing prey. It is suggested that this agitation takes the place of peristalsis in promoting digestion and absorption of the prey.

In the researches of Fenner[14] on insectivorous plants, *Sarracenia flava* was the only species of the *Sarraceniaceæ* studied. In their normal condition, the pitchers contained no liquor. Secretion of a digestive fluid occurred after insects were present in the pitcher. Flies were introduced into a young pitcher in sufficient number to fill the lowermost part of the pitcher, and were pressed together so that the mass was in intimate contact with the wall of the pitcher. In the course of 2 or 3 hours, a slight amount of mucilaginous secretion was poured out from the pitcher lining, and digested the insect bodies which were in immediate contact with the lining. The products of digestion were absorbed. Each newly captured insect shoves together the cadavers already present, and again brings them into direct contact with the pitcher lining and the secretion. The mass of insect remains therefore is well moistened and thoroughly saturated with the secretion. When the insect remains were present only in the absorptive zone, putrefaction was entirely absent. When the amount of prey was so great that the absorptive zone was filled, and a large number of insects were present in the detentive zone, then a very distinct odor of decay emanated from the latter zone; this phenomenon occurred chiefly in older pitchers.

Certain changes in the cell-contents were attributed to absorption of the

products of digestion. When cadavers of freshly captured insects were present in a pitcher, the cells in the bottommost or absorptive zone were characterized by a typical aggregation and turbidity of their contents, due to absorption of organic substances. These changes were most marked in the innermost layer of the pitcher wall. Another indication of absorption was the frequent occurrence of a large number of dark masses in the second layer of cells of the pitcher lining in the absorptive zone.

Several tests were made of the absorptive power of the pitcher. A small volume of water was introduced into a pitcher so that its level did not extend above the absorptive zone; the water was absorbed in 2 or 3 days.

When a larger volume of water was used, absorption produced a slight sinking of its level, but soon ceased. Introduction of insects into such a pitcher was followed by development of a putrefactive odor within 4 to 6 days. The dilution of the secretion with much water had unfavorably influenced the digestive function of the absorptive zone.

Several drops of meat juice were introduced into a very young pitcher; they disappeared after a time, even though the orifice of the pitcher had been closed with a cotton plug to prevent evaporation. Absorption therefore took place. However, if the pitcher were half-filled with meat juice, and the level of the latter extended far above the absorptive zone, an odor of putrefaction appeared at the end of approximately 10 days. These experiments showed that the absorptive power is limited.

Fenner concluded that *Sarracenia flava* is an insectivorous plant, provided with a digestive enzyme, requiring and utilizing its prey for its nutrition, and able to absorb animal substances through a definite, though small, region of the pitcher.

Robinson[15] conducted greenhouse experiments on plants of *Sarracenia purpurea* which had been gathered recently at Poughkeepsie, New York, and near Lakewood, New Jersey. Various substances were introduced into the pitchers, usually as aqueous solutions; and the changes in both the pitchers and the introduced substances were noted.

"Before a solution was placed in a pitcher the contents were withdrawn by means of a pipette, the pitcher was thoroughly, though gently rinsed with tap-water and distilled water, and swabbed with absorbent cotton. After the solution had been placed in the pitcher, it was covered with lace net." Insects were thereby kept out of the pitchers; an additional precaution to

exclude ants from the pitchers was to place the crocks, containing the plants, in water.

Solutions, introduced into the pitchers, produced the following results:

A 0.5 percent solution of acetic acid caused pitchers to wither above the level of the liquid within a few hours; the pitchers were dead at the end of 6 days.

A $\frac{1}{1024}$ molar solution of potassium nitrate did not injure the pitchers in which it was kept, with frequent renewals, for 6 weeks. A 0.5 percent solution of this salt was not injurious; in one experiment, perceptible growth occurred in its presence. With a 1 percent solution, the pitchers withered in 6 days; with a 2 percent solution, they became dry and brown in 3 days. Both young and mature pitchers behaved in the same manner.

Sach's nutrient solution, each liter of which contained 1.0 gram calcium nitrate, 0.25 gram potassium nitrate, 0.25 gram dipotassium phosphate, 0.25 gram magnesium sulphate, and a trace of ferrous sulphate, caused pitchers "to begin to decay within a few days, the tissues being entirely dead in from two to three weeks."

A dilute solution of Liebig's meat extract produced partial withering of the pitcher in less than a week; and complete decay occurred in approximately 2 weeks.

Milk was diluted, 1 drop in 10 cc. of distilled water; the resulting solution was neutral to litmus; it remained odorless and neutral in reaction after 6 days in the pitcher. When the concentration of the milk was doubled, acidity developed, and the pitcher decayed almost completely in 2 weeks. With a still higher concentration of milk (20 percent by volume), the pitcher contents coagulated and acquired an unpleasant odor within 2 days. "It was inferred that the pitcher gave out an alkaline substance which reacted with the acid produced in the very dilute solution of milk but was not sufficient to neutralize the solutions of greater strength. There was nothing to indicate that the milk fat or protein was digested."

When a 10 percent solution of glucose was kept in the pitchers for periods varying from 4 days to 3 weeks, it retained the power to reduce Fehling solution on heating, and the presence of much carbohydrate was shown by the α-naphthol test. Some of the glucose underwent ordinary fermentation, but the products were without apparent detrimental influence on the plant.

Sucrose was used in concentrations between less than 1 percent and 33⅓

percent; no bad effect was noted. The 33⅓ percent solution did not injure the pitcher during a period of 2 months; young pitchers containing this solution grew at the same rate as those containing distilled water. After sucrose solutions of various concentrations had been in the pitchers from 3 to 7 days, they gave a reddish precipitate of cuprous oxide with hot Fehling solution, and also spontaneously produced a heavy reduction of that reagent without heating. Blank, or control, experiments on the reagents and on water, which had been kept in the pitchers, gave no reduction. Therefore the sucrose had been cleaved into invert sugar.

Starch paste was kept in the pitchers for from 3 to 13 days: it then reduced Fehling solution, but only on boiling: and it still gave a blue color with iodine. In some experiments toluene was introduced into the pitchers with starch paste; sufficient toluene was used to form a layer above the surface of the starch paste; the contents of these pitchers also acquired the power to reduce Fehling solution. Reduction of Fehling solution was never obtained in control experiments made on the reagents, and on tap-water plus toluene which had been permitted to stand in the pitchers.

Neutral olive oil was mixed intimately with either distilled or tap water, in the ratio of 0.4 cc. of oil and 9.6 cc. of water. The mixture was introduced into pitchers; in some experiments no bactericide was used, in others toluene was added as a bactericide; the results were the same in the two series of experiments. From 4 to 7 days later, the contents of the pitchers were removed and titrated with 0.01 molar potassium hydroxide solution, using phenolphthalein as an indicator. Control experiments were made on portions of the mixture which were not introduced into pitchers. The titrations showed that the oil had not been hydrolyzed by the pitchers.

Ethyl butyrate was also used as a reagent for a fat-splitting enzyme. Tap water, or 0.01 molar potassium hydroxide solution, or 0.01 molar acetic acid solution was kept in the pitchers for 1 day. The liquid was then removed and mixed with ethyl butyrate, 4 drops of the ester to 2 cc. of the liquid. After incubation at room temperature for 24 hours, the liberated butyric acid was titrated with 0.01 molar sodium hydroxide solution, using phenolphthalein as an indicator. Control experiments were made *in vitro* with the ester and portions of the standard solutions. The results showed the absence of enzymic cleavage of the ester.

Distilled water produced no change in the external appearance of pitchers in which it was kept, with frequent renewals for approximately 5 weeks.

In another series of experiments water was kept in the pitchers for 6 days, then removed, and tested for the presence of a protease. The water from the pitchers was divided into several portions, to each of which a granule of fibrin was added. The test was carried out in neutral, in slightly acid, and in slightly alkaline solution; each of these three sets of tests was made both in the presence and in the absence of toluene. "The result was quite uniform, for the fibrin granule remained apparently unchanged in each liquid."

The general conclusions reached by Robinson were:

"1. The pitchers of *Sarracenia purpurea* can adapt themselves to solutions of very different osmotic strengths.

"2. They give out an enzyme which hydrates sucrose and starch to reducing materials, presumably simple sugars.

"3. They have no fat-digesting power.

"4. They do not secrete a protein-dissolving enzyme."

Hepburn, St. John, and Jones[18] made a preliminary report on the presence of a protease in the liquor of both unopened pitchers and open pitchers of *Sarracenia flava*, on the bacterial sterility of the contents of unopened pitchers of *S. flava* and *S. minor*, and on the types of bacteria present in the contents of open pitchers of these two species.

In another paper, Hepburn, St. John, and Jones[19] made a preliminary report on the biochemistry of the pitcher liquor of the North American *Sarraceniaceæ*, and on the response by the pitchers to introduced substances. They also gave a detailed account of the absorption of various nutrient solutions which were introduced into the pitchers.

Certain flies of the genus *Sarcophaga* pass their entire larval stage within the *Sarracenia* pitchers, living on the cadavers of the captured insects. Hepburn and Jones[20] demonstrated the presence of antiproteases in these larvæ; this indicates that the proteases, which occur in the pitcher liquor, exert a digestive action on proteins present within the pitcher cavity.

Burnett[21] wrote of the pitchers of *Sarracenia:* "The water in these receptacles, impregnated by the half-decomposing animal matter, doubtless affords a highly nutritive and invigorating diet to the plant," and "the Sarraceniæ, if kept from the access of flies, are said to be less flourishing in their growth, than when each pouch is truly a sarcophagus." He mentioned digestion of the prey, and spoke of the pitchers as special organs "for the especial purpose of retaining food, and absorbing thence its nutritious particles." He

considered the pitchers the nearest approach in the vegetable kingdom to the stomach of animals. Burnett apparently believed that the prey underwent digestion within the pitchers, and that the products of digestion were then absorbed and used for the nutrition of the plant.

Wherry[94] has commented on the reaction (hydrogen-ion concentration) of the pitcher liquor of *Sarracenia purpurea*.

Values obtained by Wherry on the pitcher liquor of this and other species of *Sarracenia* have been placed at the disposal of the authors, and are given on page 69.

PHYSIOLOGICAL ACTION OF THE PITCHER LIQUOR AND OF THE NECTAR

The Pitcher Liquor

The physiological action of the pitcher liquor on insects was studied by Mellichamp.[4] Liquor was collected from pitchers of *Sarracenia variolaris* (*S. minor*). Insects were placed in a layer of the liquor which was not deep enough to immerse them completely. House-flies were used in approximately 20 experiments; they became anesthetized or intoxicated in from 0.5 to 10 minutes. When removed from the liquor, they recovered; the time required for recovery varied from 0.5 hour to one hour or longer. The liquor also exerted this action on a cockroach, a moth, and a common house spider. "Without doubt, therefore, the secretion found in the tubes of *Sarracenia variolaris* is intoxicating, or narcotic, or anesthetic, or by whatever word we may prefer to indicate that condition to which these small insects succumb." The liquor quickly saturated, clung to, and clogged the wings of house-flies, rendering flight impossible. Pure water does not exert this wetting action, but "runs" from the wings.

The following experiment showed that the pitcher liquor does not evolve a poisonous exhalation for overcoming the prey. Two house-flies were suspended in a cage of thin gauze within a large wide-mouth phial, which contained approximately one-half ounce of pitcher liquor; about 8 hours later, the flies were still struggling frantically to escape.

In a second communication on the stupefying action of the pitcher liquor of *Sarracenia variolaris* (*S. minor*), Mellichamp[16] wrote: "Pour out a teaspoonful or two of the fluid in an ounce measure or a small wine-glass.

Throw in a fly so that his wings will be wet or slimed. He will in a few minutes cease to struggle and will appear as if dead. Take him out after a while and let him dry, and in about half an hour he will revive."

Pitcher liquor was collected at a time when no rain had fallen for nearly two weeks, and, in part, from unopened pitchers; it was turbid, slightly acid in reaction, had very little, if any, taste, and was very active with respect to stupefying power.

A sample of pitcher liquor, which had been kept for 3 years, was clear, neutral in reaction, and without much sediment; it was nearly or quite inert with respect to the stupefying power.

Watson[17] repeated the experiments of Mellichamp, and confirmed the latter's results on the stupefying power of the pitcher liquor of *Sarracenia variolaris* (*S. minor*).

Zipperer[11] conducted experiments on the action of the pitcher liquor of *Sarracenia purpurea* on isopods of the genus *Oniscus*, and concluded that the liquor first exerted a narcotic action on the isopods, and that their death was due to suffocation.

From experiments made by Lambert[13] on millipeds, it appeared that the pitcher liquor of *Sarracenia purpurea* had no action on *living* animal organisms.

The Nectar

Mellichamp[4] permitted flies, large red ants, and smaller ants—both black and red—to feed on the nectar of *Sarracenia variolaris* (*S. minor*). The nectar was innocuous, and entirely without narcotic, stupefying, or intoxicating action on these insects.

This conclusion was confirmed by later experiments of Mellichamp,[16] made on pitchers (leaves) of this species. "While still fresh, the upper portions of these leaves were cut off and slit open, thereby exposing the honeyed secretion on the internal surface, which was very abundant and glistening, sweet to the taste and viscid to the touch. These were then flattened out on a large newspaper, the whole surface of which was covered with them. Many house flies were soon attracted and commenced to feed, and I carefully watched their motions without any interruption for the space of one hour. The result was precisely as previously stated. In no instance did I discover the slightest unsteadiness or tottering in any of the flies, although I watched some of them

feeding at one spot for at least ten minutes, at the expiration of which time they flew off apparently unhurt. They continued feeding and flying off from the leaves during the hour I watched them, and certainly not one fell, nor was there any indication at any time of either stupor or intoxication." The pitchers, which were used, were secreting nectar freely. The experiment was repeated twice, and the same result was always obtained. The nectar functioned simply as a lure.

CHEMICAL COMPOSITION OF THE TISSUES

Porcher[23] made various qualitative tests upon the cold infusion, the decoction, and the alcoholic extract of the rhizomes of *Sarracenia flava* and *S. variolaris* (*S. minor*). Meconic acid, morphine, narcotine, and quinine were not found in the rhizomes. Volatile, bitter and astringent principles are mentioned: "The bitter and astringent principles are volatile, for upon boiling a portion of the powdered root, the liquid was almost tasteless."

Porcher included in his paper the results obtained by Shepard in a preliminary examination of the dried rhizomes of the species mentioned. Sections of the rhizomes imparted an acid reaction (red color) to moistened blue litmus paper. Upon incineration, a white ash was obtained containing silica, calcium carbonate, and potassium carbonate. From qualitative tests, made chiefly on the extract in 80 percent alcohol and the extract in water acidulated with sulphuric acid, Shepard concluded that the rhizomes contained liguin, coloring matter, traces of a resin, an acid salt of calcium "(the acid being neither the tannic nor the gallic, but possibly one altogether new), and a salt of some alkaloid, related perhaps to cinchonia, which, should it prove new, may be called Sarracenia."

Both Porcher and Shepard noted the pigment. Shepard obtained a rich red precipitate (color of port wine) on the addition of potassium carbonate to the solution obtained by extraction of the rhizomes with water acidulated with sulphuric acid. The color was attributed to the pigment of the rhizomes. Porcher described the extraction of pigment from the rhizomes by spirits of ammonia with the production of a solution of blood red hue.

Björklund and Dragendorff[24, 25] made an exhaustive study of the chemical composition of the dried tissues of *Sarracenia purpurea*. The rhizomes and the pitchered leaves were studied separately. The various chemical compounds were separated from each other and then identified by appropriate

tests; and quantitative determinations were made of the amount of each compound present. The specimens examined apparently had been collected in the spring of the year.

The percentage composition of the tissues, as reported by these investigators, is given in the table. In addition to the compounds there enumerated, they state that the following substances were present in quantities "not determinable":

PERCENTAGE COMPOSITION OF THE DRIED TISSUES OF *SARRACENIA PURPUREA* ACCORDING TO BJÖRKLUND AND DRAGENDORFF

Constituents	*Rhizomes*	*Pitchers (Leaves)*
Hygroscopic moisture	12.08	8.60
Cellulose	19.82	14.55
Starch	25.55	..
Lignin, cuticular substance, and insoluble plant mucilage	3.17	19.90
Soluble plant mucilage	0.89	..
Sugar	9.56	3.95
Soluble plant albumin	5.70	1.02
Insoluble plant casein	7.10	1.40
Volatile amide	0.18	0.77
Volatile acid (acrylic acid)	1.49	0.12
Indifferent white resin	8.81	5.47
Wax	0.10	0.53
Ash	2.25	2.14
Silica	0.21	0.31
Ferric and phosphoric oxides	0.91	0.50
Calcium carbonate	0.09	..
Potassium sulphate	0.25	0.64
Sodium chloride	0.45	0.03
Magnesium carbonate	0.03	..
Sodium sulphate	0.15	..
Calcium sulphate	...	0.71

In the *rhizomes:* (1) an unknown substance which, on boiling in aqueous solution, furnishes a substance similar to cinchona red; (2) a non-volatile acid; (3) a tannic acid similar to caffetannic acid; (4) amorphous extractives; (5) a chromogen which yields a red pigment on treatment with hydrochloric acid, and (6) a volatile aromatic substance with an odor similar to that of "Rad. Carlinæ."

In the *pitchers (leaves):* (1) a non-volatile acid; (2) tannic acid; (3) plant mucilage soluble in boiling water; (4) non-crystalline extractives; (5) a red

pigment soluble in dilute hydrochloric acid, and (6) traces of carbonates and magnesium.

The volatile amide, which occurred in both the rhizomes and the pitchers, is also termed an alkaloid, but lacked the toxic properties of a true alkaloid as was shown by feeding it on bread to a mouse. It was a liquid at ordinary temperatures; its molecular weight was approximately 35; its odor resembled that of coniine; and it formed a hydrochloride.

In order to ascertain the physiological action of the volatile amide, approximately one centigram of its hydrochloride was dissolved in a very little water, absorbed in about one-half a gram of white bread, and given to a mouse. The mouse ate approximately one-half the mass of moistened bread greedily, then gradually stopped eating, and later showed no inclination to consume the remainder of the bread. In about one-half an hour, the animal became indolent, and remained so for about 20 minutes, then again behaved in a normal manner; these attacks of dullness and indolence returned several times. The mouse showed no inclination to drink, and now and then ate white bread, but refused to consume the remainder of the bread which had been treated with the hydrochloride. It urinated very frequently. Death occurred after 12 hours. Autopsy showed almost complete emptiness of the heart, marked extravasation of blood in the lungs, and slight quantities of blood in the brain. The stomach contained some viscous black material adhering to its walls. The intestines contained black feces alternating with gas; the gas was most abundant in the uppermost parts of the intestines. The bladder was completely filled. Björklund and Dragendorff decided that, while this single experiment did not permit a definite conclusion concerning the toxicological action of the volatile amide, yet it supported the view that this compound does not belong among the true poisonous alkaloids, and also indicated that the diuretic action of the rhizome may be due, at least partly, to the presence of this amide.

Both the rhizomes and the pitchers contained the same resin; but the chlorophyll content of the pitchers was included in the percent of resin reported for them.

The species of sugar present in the tissues was not determined.

The substance, similar to cinchona red, derived from the rhizomes, was soluble in alkalies with a red color.

The red pigment present in hydrochloric acid decoctions of the rhizome

was apparently derived from a chromogen occurring in the cambium layer, which dissolved readily in water, very difficultly in alcohol, and was converted into the pigment by cleavage by the acid. It was precipitated from its solution in hydrochloric acid as a violet pigment on addition of ammonia.

A proximate analysis of *Sarracenia purpurea* by Frohwein is included in a report on that plant by a committee of the New York County Medical Society.[26] Since the report deals with the use of the rhizome as a remedy for small-pox, rhizomes probably were the tissues analyzed. Frohwein reported the presence of gum, starch, vegetable albumin, tannin, resin, bitter principle with an acid reaction, extractive matter, traces of volatile oil, calcium, magnesium, potassium, sodium, iron, silica, phosphoric acid, sulphuric acid, and carbonic acid. "From this analysis it would seem that an alkaloid does not exist in the root of the *S. purpurea*, and it might be considered only a mild tonic, on account of the bitter principle which it contains."

Martin[27,28] found in the rhizomes of *Sarracenia purpurea* an alkaloid (sarracenine), a resin, a yellow coloring matter, extractives, and substances which form the skeleton of plants.

In order to obtain the alkaloid, sarracenine, the rhizomes were pulverized and made into a thin paste with distilled water acidified with sulphuric acid. This paste was kept in an oven until desiccation was complete. The powdery residue was extracted for four days in a flask with carbon disulphide, with agitation from time to time. The supernatant liquid was removed by filtration through linen under strong pressure. The woody residue on the filter contained the sarracenine; it was extracted with boiling distilled water in a capsule for one-half hour; the resulting solution was removed by filtration under strong pressure. The filtrate was again filtered, then evaporated on the water-bath to a syrupy consistency. This syrup was mixed with twice its volume of ethyl ether in a flask which was shaken vigorously at intervals for 1 or 2 days; the ether was then decanted, and permitted to evaporate spontaneously. The residue was dissolved in distilled water. The resulting solution was filtered if necessary, and was concentrated on the water-bath until the sarracenine sulphate crystallized. Pure sarracenine was obtained by mixing its sulphate with sodium bicarbonate, and isolating the free alkaloid by means of rectified alcohol.

Sarracenine is described as a white substance, soluble in alcohol and ether, and forming salts with acids. Its sulphate crystallizes in beautiful needles, and has a bitter taste which it imparts to its solutions.

Reference is at times made in the literature to an abstract of Martin's paper.[29]

Schmitt[30] carried out a series of experiments on dried *entire plants* of *Sarracenia purpurea*. They contained 11.43 percent of hygroscopic moisture (determined by drying at a temperature of 120° C.), and yielded 3.32 percent of a white ash. Analysis of the ash showed the presence of much potassium, also of calcium and the radicals of the following acids: sulphuric, phosphoric, silicic, and hydrochloric.

He speaks of the use of the decoction and the tincture of these plants in pharmacy, and of the occasional use of the powdered plant. The decoction was prepared by mixing 50 grams of the plant with 1 liter of water, and concentrating to a volume of 500 cc.; it had a brownish yellow color; the dose is given as one-half glass in 24 hours. The tincture was prepared by percolation or maceration of 1 part of the plant with 5 parts of 80 percent alcohol; it was green in color. Both the decoction and the tincture were acid to litmus; they were subjected to qualitative tests for the presence of various compounds.

As a result of his experiments, Schmitt reports the occurrence in the plant of the following substances: (1) Plant skeleton, (2) gums, (3) albuminous substances, (4) resins, (5) sarracenic acid, (6) sarraceno-tannic acid, (7) fats and waxes, (8) potassium and calcium salts of organic acids, (9) inorganic compounds, and (10) water.

Sarracenic acid is the name given to the coloring matter of the plant. It is described as almost, if not entirely, insoluble in water, ether, and petroleum ether, and dissolves most readily in alcohol. It is an acid with a bitter taste; and its compounds with the alkalies and alkaline earths have a characteristic yellow color. The yellow solution obtained by the action of the alkaline earths (lime or baryta) upon the tincture of the plant reacts with alum to form, as a precipitate, a beautiful yellow color-lake, while the filtrate from this precipitate is entirely colorless.

Sarraceno-tannic acid is described as a physiological tannin, belonging to the group of tannins which includes those occurring in coffee, catechu, and Peruvian bark.

When the dried entire plant was subjected to distillation with water, volatile products were not obtained.

Vines[31] made a study of glycerol extracts of the pitchers of *Sarracenia flava*. Some pitchers were extracted with glycerol without previous treatment

with acid; others were treated with 1 percent acetic acid for 24 hours in order to activate a zymogen (if present), then were extracted with glycerol. These glycerol extracts were then tested for the presence of a protease. A portion of the glycerol extract, a fragment of swollen fibrin, and 2 cc. of 0.2 percent hydrochloric acid were mixed and permitted to digest at a temperature of 40° C. The period of incubation is not stated, but probably was 6 or 8 hours. In this test, a partial or complete solution of the fibrin and a positive biuret reaction, yielded by the filtered digestion-fluid, were considered evidence of the presence of a protease in the glycerol extract. Vines found no evidence of the presence of a protease in the tissues of the pitchers of *Sarracenia flava*, although he was able to demonstrate the presence of a protease in the tissues of the pitchers of certain species of *Nepenthes* by means of this technic.

The glycerol extract of the pitchers of *Sarracenia flava* was found to contain sugar.

Hetét[32] examined "powder of the leaves," therefore dried pitchers, of *Sarracenia purpurea* from the isles of Saint Pierre and Miquelon. He found in them "an alkaline substance, whose properties are identical with those of veratrine. The crystallization is the same, in beautiful prisms and in octahedra of the orthorhombic system. It behaves in the same way with the principal neutral solvents; it gives the same reactions with the acids and the solutions used to distinguish the alkaloids, that is, particularly, the successive colorations with concentrated sulphuric acid, with sulphomolybdic acid, and especially hydrochloric acid and heat, which produce that beautiful, persistent, reddish violet coloration, quite peculiar to veratrine."

Hetét also found in the pitcher the amine previously discovered by Björklund and Dragendorff,[24, 25] and "another alkaline substance, soluble in water."

Lambert[13] reports experiments, apparently made on fresh plants of *Sarracenia purpurea* on the isle of Saint Pierre and on the coast of Newfoundland. He studied sarracenic acid, the yellow coloring matter of the plant, which had been observed previously by Schmitt.[30] Lambert writes: "We have very easily recognized this coloring matter in the plant and have isolated it. We have ascertained that it has the singular property of being very soluble in alcohol, which it slightly colors. It suffices to add several drops of an acid (nitric acid seems to serve best) for the alcoholic solution to acquire a beautiful red color. On the other hand, several drops of any alkali

make the red color pass, after neutralization, to a dull green; and the least trace of an acid suffices to restore the color to its original red. This coloring matter, then, indeed behaves like a veritable acid and compares singularly in its reactions with litmic acid. The sensitivity of the coloration of this acid is such that it has appeared to us as capable of replacing litmus or phthalein in the titration of alkalies and of acids."

Lambert also tested the plants for the presence of a volatile base. A tiny fragment of the plant was treated with a dilute potash solution in a test-tube. When the tube and its contents were heated, the characteristic mouse-like odor of coniine was at once recognized.

The text does not state whether these experiments on the pigment and the volatile base were made on the pitcher, the rhizome, or the entire plant. However, attempts were made to isolate the base from the leaves (pitchers), but were fruitless; for the base was present in so small an amount that only the odor was obtained. It is suggested that this base acts as a lure for insects.

Gies[33] used fresh pitchers of *Sarracenia purpurea* in his experiments. The pitchers were thoroughly macerated in glycerol; and the resulting extract was used in tests for the presence of a protease. The glycerol extracts obtained from one set of plants showed moderate but distinct digestive action on fibrin at a temperature of 38° C. in the presence of "slight amounts of hydrochloric or oxalic acids"; the control experiments showed no digestion. The glycerol extracts from a second set of plants entirely lacked digestive action. Gies interprets these results: "In view of the negative results in the second series it is impossible at present to draw a satisfactory conclusion in this connection. It may be that the positive results in the first case were due to a bacterium specially favored by the medium furnished by the constituents of the glycerin extract, or to enzyme in unobserved diseased portions of the plants. Again, the negative results may have been due to a less favorable degree of acidity, or the secreting cells of the pitchers may have been in a 'resting condition' without either enzyme or zymogen." The two sets of plants came from different localities.

The diluted glycerol extract was practically colorless when neutral, crimson with acids, and green with alkalies. Further experiments showed that "Sarracenia purpurea contains a pigment which in concentrated glycerin extract has a reddish color, but which when diluted is practically colorless. At such dilution, when scarcely any color is to be seen, a drop of dilute acid

produces a bright pink throughout the whole fluid; alkali in minute amounts turns it green. The pink is converted to green by alkali, *vice versa* by acid. Even in crude glycerin extract the pigment appears to be very sensitive and may be used to advantage in titrimetric work. I have named the pigment alkaverdin, because of the beautiful green produced on treatment with alkali. . . . Excellent 'test papers' have been made with the pigment in glycerin extract. Ordinary filter paper dipped into the red, concentrated extract is colorless wet or dry. The dry paper turns a bright pink when dipped into acid, a deep green is produced when in contact with alkali." The solutions of alkaverdin had no special influence on the spectrum.

Both the aqueous and the saline extracts of the pitchers contained "an abundance of dextrorotatory, reducing and fermentable substances."

Meyer and Gies[34] found that alcohol extracted 3 coloring matters from macerated purple pitchers of *Sarracenia purpurea:* green chlorophyll, purplish red alkaverdin, and a brownish black substance. Water extracted only alkaverdin and the brownish black pigment from the pitchers. When the aqueous extract was evaporated almost to dryness *in vacuo* and was then poured into absolute alcohol, the brownish black pigment was precipitated. The color of this pigment was not influenced by either acid or alkali; it did not react with ferric chloride, and had no reducing power.

The alcoholic solution now contained all the alkaverdin; it was evaporated almost to dryness *in vacuo*. The residue had the color and consistency of dark molasses, a bitter taste, and an odor which was sugary and also resembled the characteristic odor of the macerated pitchers. It was insoluble in ether and chloroform, but soluble in water and alcohol, was free from the halogens, nitrogen, and sulphur, and contained a large amount of fermentable dextrorotatory carbohydrate which had a marked reducing action on Fehling solution and yielded a phenyl osazone resembling in crystal form that derived from glucose (dextrose). Removal of the sugar by fermentation had no apparent injurious action on the alkaverdin.

The aqueous solution of this molasses-like syrup had a reddish color, but became colorless when very dilute. Such a colorless solution became green on addition of a drop of 0.2 normal alkali, and returned to its colorless condition on addition of a drop of 0.2 normal acid. A drop or two of the acid in excess produced a pink color, quite different from the color of the syrup, and intensified

somewhat on standing. These changes in color were due to the presence of alkaverdin; and delicate test papers were made with that pigment.

Alkaverdin was not obtained in the crystalline state; it was found to have no influence on the spectrum. When its aqueous solution was warmed for some time on the water bath, the alkaverdin was converted into a brownish substance which still gave a green color with alkali, but did not yield a pink color with acid. Hydration with 2 percent sulphuric acid caused alkaverdin to lose completely its tinctorial properties.

Clark,[35] in the course of a research on plant oxidases, examined the "pitcher-plant leaves" of *Sarracenia Drummondi*, using an aqueous extract of the fresh pitchers for his experiments. The extract did not contain *oxygenase;* presence of *peroxidase* was doubtful; *catalase* was present; *chromogens*, which are oxidized to colored compounds by the natural oxidase of the plant, were not found.

For oxygenase (direct oxidase) seven reagents were used: tincture of guaiac, tincture of guaiac previously boiled with bone black to remove peroxides, α-naphthol, 1,4 phenylenediamine hydrochloride, phenolphthalein, phenol, and the indophenol reagent (α-naphthol plus 1,4 phenylenediamine hydrochloride in the presence of sodium carbonate). Hydrogen peroxide was added to each of these reagents in testing for peroxidase. Catalase was detected by its action in liberating molecular oxygen from a dilute solution of hydrogen peroxide.

A CHEMICAL STUDY OF THE NECTAR OF THE SARRACENIACEÆ

By JOSEPH SAMUEL HEPBURN, A.M., B.S. in Chem., M.S., Ph.D.

Either 25 or 50 pitchers of a given species were collected in the field while secreting nectar. They were cut from the plant near the base of the pitcher, and their tops were washed in succession with the same portion of distilled water (approximately 75 cc.). In this way an aqueous solution of the nectar was obtained free from pitcher liquor, prey, and plasma of the plant cells. The solution was filtered through filter paper to remove any insoluble particles, chiefly dust. The solution was immediately tested, by means of Benedict qualitative alkaline copper solution, for the presence of reducing sugar, and was then preserved by addition of sufficient trikresol to render the concentration of that bactericide 0.2 percent. Further tests were made in the laboratory at Philadelphia.

Separate series of tests were made on each of the following species: *Darlingtonia californica*, *Sarracenia minor*, *S. Sledgei*, *S. flava*, *S. Drummondii*, and *S. purpurea*.

The aqueous solution of the nectar of each of these species gave the following reactions:

(1) It reduced Benedict qualitative alkaline copper solution, showing the presence of a reducing sugar.

(2) It yielded the characteristic crystals of phenylglucosazone in the osazone test. This test was performed in two ways. The aqueous solution of the nectar was digested at the temperature of boiling water with phenylhydrazine hydrochloride and sodium acetate, then permitted to stand at the temperature of the room. Or the aqueous solution of the nectar was heated for one minute over a low flame after the addition of free phenylhydrazine and acetic acid; the solution was rendered almost, but not quite, neutral by addition of an approximately 14 percent aqueous solution of sodium hydroxide, was again heated for 1 minute, then permitted to stand at the temperature of the room. In either case crystals of an osazone were formed, and were identified as those of phenylglucosazone by microscopic examination.

(3) It gave a distinct crimson color in the Seliwanoff test. In this test, a portion of the aqueous solution of the nectar was mixed with its own volume of Seliwanoff reagent and then heated at the temperature of boiling water for exactly 20 minutes. The Seliwanoff reagent was an aqueous solution containing 0.05 percent resorcinol and approximately 12 percent hydrochloric acid. The development of the crimson color under the conditions of the test was evidence of the presence of a ketose sugar.

These reactions indicated that the nectar contained either fructose (levulose) or invert sugar.

The aqueous solutions of the nectar of *Darlingtonia californica* and *Sarracenia flava* were sufficiently concentrated to permit quantitative examination, both chemically and optically. In the *chemical* examination, the solution was titrated against Benedict quantitative alkaline copper solution to determine its reducing sugar content which was then calculated as glucose. This Benedict solution was standardized by titration with a freshly prepared solution of glucose of the highest purity. For the *optical* examination, the solution of the nectar was first clarified by addition of solid lead subacetate (Horne), thorough mixing, and filtration through a dry filter paper; this treatment removed all optically active compounds other than sugars. The filtrate was treated with solid neutral potassium oxalate to remove the excess of lead; and the resulting solution was filtered through a dry filter paper. This filtrate was used for the optical examination in a Peters saccharimeter.

The solution of the nectar of *Darlingtonia californica* was found, by the Benedict method, to contain 0.22 percent of reducing sugar, calculated as glucose. Examined in a standard tube, 200 mm. in length, it gave a reading of $-0.9°$ in the saccharimeter.

According to Browne,[69] the relative copper reducing values of the sugars in the volumetric method are:—Glucose 1.000, fructose 0.924, invert sugar 0.962. This author gives the following normal weights of sugars for saccharimeters with a Ventzke scale:—Fructose 18.592 grams, invert sugar 86.450 grams.

From the relative copper reducing values $\frac{\text{Glucose}}{\text{Frutcose}} = 0.924$ and $\frac{\text{Glucose}}{\text{Invert Sugar}} = 0.962$, it follows that the same copper reducing value is possessed by solutions containing 0.22 percent glucose, 0.24 percent fructose, and 0.23 percent invert sugar respectively. Therefore, the aqueous solution of the nectar contained either 0.24 percent fructose or 0.23 percent invert sugar.

From the normal weights, it follows that a solution producing a rotation of −0.9 saccharimeter degree may contain 0.009 times 18.592 or 0.17 gram of fructose in 100 cc., *i. e.*, 0.17 percent fructose. Or it may contain 0.009 times 86.450 or 0.78 gram of invert sugar in 100 cc., *i. e.*, 0.78 percent invert sugar.

Since the values for fructose, obtained by copper reduction (0.24 percent) and by the optical method (0.17 percent), show a far closer agreement with each other than do those for invert sugar (0.23 percent by copper reduction and 0.78 percent by the optical method), it follows that the reducing sugar present in the nectar of *Darlingtonia californica* is fructose (*d*−fructose, levulose) and not invert sugar.

The solution of the nectar of *Sarracenia flava* was found, by the Benedict method, to contain 0.17 percent of reducing sugar, calculated as glucose. Examined in a tube 400 mm. in length, it gave a reading of −1.4° in the saccharimeter; this corresponded to a reading of −0.7° for a standard tube 200 mm. in length.

From the relative copper reducing values, it follows that the same reducing value is possessed by solutions containing 0.17 percent glucose, 0.18 percent fructose, and 0.18 percent invert sugar, respectively.

From the normal weights, it follows that a solution producing a rotation of −0.7 saccharimeter degree may contain 0.13 percent fructose or 0.61 percent invert sugar.

Since the values for fructose, obtained by copper reduction (0.18 percent) and by the optical method (0.13 percent), show a far closer agreement with each other than do those for invert sugar (0.18 percent by copper reduction and 0.61 percent by the optical method), it may be concluded that in *Sarracenia flava* also, the reducing sugar present in the nectar is fructose and not invert sugar.

OBSERVATIONS ON THE PITCHER LIQUOR OF THE SARRACENIACEÆ

By FRANK MORTON JONES, F.E.S., and
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Volume of the Normal Pitcher Secretion

The pitchers of all North American *Sarraceniaceæ* grow to a considerable size and even approach their full size before the orifice of the pitcher opens. These hermetically sealed or closed pitchers normally contain a larger or smaller volume of clear fluid which is transparent and colorless, though sometimes rendered slightly milky by suspended particles. The volume of the fluid or pitcher liquor is much greater in large vigorous pitchers than in small stunted ones in which the liquor may be almost entirely absent. In *Sarracenia purpurea* and *S. psittacina*, the quantity of pitcher liquor is very small; even in the larger pitchers, it usually appears only as a studding of minute perspiration-like drops on the lower wall and rarely collects in the bottom of the cavity. In all the other species, the pitcher liquor occurs in sufficient quantity to be drawn off with a pipette, and may fill the narrow tube to a height of several inches in vigorous pitchers.

After opening, the pitchers of *Sarracenia purpurea* usually become filled, entirely or partly, with rain water. Certain species, especially *Sarracenia purpurea* and *S. psittacina*, may be partially or completely submerged in time of flood as a result of their shape, size, and habitat. Beating rains may enter, in greater or less degree, the pitchers of the remaining species of *Sarracenia*. The level of the liquid contents of the pitcher, thus augmented, may be reduced again by evaporation or absorption. In dry weather, and especially when the soil about the roots becomes dry, the pitchers may be found to have lost much or all of their liquid contents. Under these varying circumstances, the contents of an open pitcher may consist principally of a mass of insect remains ranging from dry and friable to moist and broth-like, or of insect cadavers bathed in a liquid which may range from clear to markedly turbid, or of insect captures floating in an excess of liquid (rain water or flood water) which, necessarily, highly dilutes the secretion of the pitcher.

The volume of the pitcher liquor, reported in Table I for *Darlingtonia*

californica, *Sarracenia Sledgei*, *S. flava*, and *S. Drummondii* are based on measurements of the volume present in normal, full-sized pitchers—closed, plugged, or open—of these species growing in favorable localities at the height of their season and not materially affected by external conditions. The volumes recorded represent the amount of secreted liquid present in the pitchers at the time of examination. *Closed* pitchers were used, as nearly as possible, at their point of opening. *Plugged* pitchers, in age and development, were open and active but free from insect captures. The top of a closed pitcher, which was about to open, was folded over; a piece of paper bearing the date was included in the fold; and one or more pins were passed through the doubled pitcher and paper, effectually sealing the top. The pitcher did not wither, but continued to grow and to expand its top even above the fold; it was permitted to remain in this condition for 1 week in the case of the Sarracenias and for as long as 1 month in the case of *Darlingtonia californica*. *Open* pitchers were fully mature, and actively engaged in the capture of insects.

Determination of the volume.—The upper portion of the pitcher was removed with scissors until the pitcher liquor was within reach of a plain pipette, the lower portion of which was of fine bore. As each portion of the liquor was removed by means of the pipette, another section of the pitcher was cut off. This procedure was repeated until the entire liquid contents of the pitcher had been collected. The total liquid contents of a definite number of pitchers were collected in a stoppered vial by transfer from the pipette. The total volume obtained from the lot was measured in a narrow graduated cylinder; and the average volume per pitcher was calculated.

Not many pitchers of *Sarracenia rubra* were obtainable. The pitchers of *Sarracenia minor* were of a size far below the recorded maximum for this species. Therefore quantitative measurements of the volume of liquor present in the pitchers of these two species have not been reported. No records are included for *Sarracenia psittacina* since the pitchers of this species do not secrete liquor in measurable quantity during their seasonal history. The liquid ordinarily present in open pitchers of *Sarracenia purpurea* is the secretion of the pitcher highly diluted by rain water, for the shape of the pitcher of this species readily permits the entrance of falling rain; therefore no measurements were made of the volume of liquor present in such pitchers. The amount of liquor present in closed pitchers of this species is so slight that its volume is not measureable.

Observations and measurements made on many individual pitchers, as well as the general results presented in Table I, indicate that the pitcher liquor progressively increases in volume as the pitchers develop. A further marked increase in the volume of the pitcher liquor occurs after the lid and lips have opened, and a still further increase is usually apparent after the capture of prey.

On account of the shape of the pitchers and the habitat of the plant, the volume of the pitcher liquor in *Darlingtonia californica* is less influenced by external conditions than in the Sarracenias. The progressive increase in the volume of the pitcher liquor is shown by the following data. As reported in Table I, 82 *plugged* pitchers, studied in 4 lots during June and July, contained, on the average, 2.50 cc. of liquor per pitcher. A single lot of 25 pitchers, plugged in June and studied during the following September, in the same bog as the other lots, contained, on the average, 3.38 cc. of liquor per pitcher. As reported in Table I, 161 *open* pitchers, examined in 5 lots during June and

TABLE I.—AVERAGE VOLUME OF THE LIQUOR IN THE PITCHERS OF CERTAIN SARRACENIACEÆ.

	Closed Pitchers			*Plugged Pitchers*			*Open Pitchers*		
Genus and Species.	*Number of lots measured.*	*Total number of pitchers.*	*Average volume per pitcher, cc.*	*Number of lots measured.*	*Total number of pitchers.*	*Average volume per pitcher, cc.*	*Number of lots measured.*	*Total number of pitchers.*	*Average volume per pitcher, cc.*
Darlingtonia californica	10	353	1.08	4	82	2.50	5	161	2.80
Sarracenia Sledgei	4	325	0.25	2	31	0.38	3	74	0.38
Sarracenia flava	6	566	0.66	1	8	1.30	4	160	1.50
Sarracenia Drummondii	4	230	0.44	1	20	0.66	4	138	0.58

July, contained, on the average, 2.80 cc. of liquor per pitcher. A single lot of 25 open pitchers, examined during the following September in the same bog as the other lots, contained, on the average, 4.04 cc. of liquor per pitcher.

The volume of pitcher liquor varies widely in any given species with the size, vigor, and degree of development of the pitcher. Thus in the 10 lots of *closed* pitchers of *Darlingtonia californica*, which were strictly comparable with each other, the maximum average volume of pitcher liquor for any lot was 1.60 cc. per pitcher, while the general average for all 10 lots was 1.08 cc. per pitcher. Also in the 5 lots of *open* pitchers of this species, likewise strictly comparable with each other, the maximum average volume of pitcher liquor for any lot was 3.61 cc. per pitcher, while the general average for all 5 lots was

2.80 cc. per pitcher. Individual pitchers of unusual size or vigor frequently contained pitcher liquor far in excess of the maximum average volumes just reported.

Reaction of the Pitcher Liquor

The reaction of the pitcher liquor to litmus was determined for the species mentioned below, using liquor from individual pitchers and composite samples of liquor from many pitchers. The mode of collection of the pitcher liquor, described above, yielded a liquid uncontaminated by the plasma of the pitcher tissues. Blue and red litmus paper (Squibb) was used; this reagent is sensitive to 0.004 normal acid and alkali respectively.

TABLE II.—REACTION OF THE PITCHER LIQUOR TO LITMUS

Genus and Species.	*Closed Pitchers.*	*Plugged Pitchers.*	*Open Pitchers.*
Darlingtonia californica	neutral	neutral	neutral
Sarracenia minor	neutral		neutral to faintly acid
Sarracenia Sledgei	acid	usually neutral	neutral
Sarracenia flava	acid		neutral to faintly acid
Sarracenia Drummondii	markedly acid	usually neutral	neutral
Sarracenia rubra	slightly acid		

The reaction of the pitcher liquor showed variations in the individual plugged pitchers of two species. In the case of *Sarracenia Sledgei*, the liquor was neutral in 20 pitchers, acid in 3 pitchers, and faintly alkaline in 2 pitchers. In the case of *Sarracenia Drummondii*, the liquor was neutral in 13 pitchers, acid in 5 pitchers, and faintly alkaline in 2 pitchers.

The change from an acid to a neutral reaction, which occurs in the pitcher liquor of several species upon the opening of the pitchers, does not depend upon or result from the capture of prey, for the same change occurs in plugged pitchers from which insects are carefully excluded.

Mechanical Stimulation

In a series of experiments on mechanical stimulation of the pitchers, efforts were made to simulate the struggles of entrapped insects by dangling strings of glass beads, gentle scratching with elastic wire brushes, etc. These experiments were made on *Darlingtonia californica*, *Sarracenia Sledgei*, *S. flava*, and *S. Drummondii*, using pitchers of all ages, closed, just open, and open active pitchers both with and without prey. The period and degree of stimulation and also the elapsed time between stimulation and withdrawal of the pitcher contents were varied. However, no measurable effect upon the volume of the pitcher liquor was detected in any of the experiments.

Food Stimulation

Reference has been made to the work of Mrs. Austin[7] on the response of *Darlingtonia californica* to food stimulation. An account of many additional experiments on such stimulation is given by her in her unpublished letters and manuscript journal, which are preserved among the Canby Papers deposited in the library of the Society of Natural History of Delaware at Wilmington.

In our experiments, use has been made of milk, beef broth, meat, other foods of animal origin, and inorganic salts.

Milk.—Fresh skim milk was diluted with its own volume of water, sterilized by heat, and permitted to cool to the temperature of the atmosphere. Measured volumes of this milk were introduced into the pitchers by pouring from a narrow graduated cylinder. With closed pitchers of the Sarracenias, with plugged pitchers of this genus, and with all three types of pitchers of *Darlingtonia californica*, the top of the pitcher was cut off just beneath the lips or pitcher rim; the milk was introduced; the uppermost portion of the pitcher was folded over; a piece of paper bearing a memorandum of the experiment was included in the fold; one or more pins were passed through the doubled pitcher and paper, effectually sealing the pitcher cavity. With open pitchers of the Sarracenias, the milk was introduced through the open mouth of the pitcher which was then sealed in the manner just described.

At the end of a given period of time, the volume of the liquid contents of the pitcher was determined in the manner already described. When more than one pitcher was used in an experiment, the average volume of liquid contents per pitcher was determined at the end of the period of stimulation. The "Percentage increase above normal liquid contents" was calculated as follows. From the average volume of liquid contents per pitcher at the end of the period of stimulation was subtracted the sum of the volume of milk introduced plus the average volume of the liquid contents per pitcher in pitchers of the same type and species, using the values given in Table I. The remainder, which represented the volume of liquid secreted by the pitcher in response to stimulation, was divided by the average volume of the liquid contents per pitcher in pitchers of the same type and species as recorded in Table I. The quotient was multiplied by 100. The final arithmetical result expressed the volume of the liquid produced by stimulation as percent of the volume of liquid usually present in pitchers of the type and species used in the experiment. The average values in Table I were used, since the determinations

reported in that table and the stimulation experiments were made at the same time and place for a given species.

TABLE III.—RESPONSE OF THE PITCHERS OF CERTAIN *SARRACENIACEÆ* TO STIMULATION BY MILK.

Genus and Species	*Number of pitchers.*	*Type of pitcher.*	*Volume of milk introduced into each pitcher.*	*Period of stimulation. Days.*	*Percentage increase above normal liquid contents.*
Darlingtonia californica	7	closed	1	1	20
" "	12	"	3	1	86
" "	8	"	3	1	38
" "	6	"	3	3	200
" "	4	"	3	6	401
" "	1	"	12	6	1242
" "	7	plugged	1	1	37
" "	6	"	1	3	63
" "	3	"	15	6	532
" "	1	"	5	7	476
" "	4	"	12	7	446
" "	6	open	3	3	57
" "	1	"	15	5	292
" "	2	"	30	5	430
" "	6	"	3	6	119
" "	1	"	15	6	328
" "	1	"	45	6	435
" "	1	"	10	10	471
" "	1	"	30	10	578
Sarracenia Sledgei	4	plugged	5	5	952
" *flava*	4	closed	10	4	1096
" "	3	open	10	4	268
" *Drummondii*	4	plugged	5	5	506

The results, which have been summarized in Table III, demonstrate that the pitchers of the species studied poured out additional pitcher liquor in response to their stimulation by the introduced milk. The volume of additional liquor thus secreted depended in part on the volume of milk introduced, and in part on the period of time during which the stimulus was permitted to act.

When 30 cc. or more of milk were introduced into a pitcher, it became necessary to support the pitcher.

In all the experiments on food stimulation, injury to the pitcher was observed in but one experiment, which has not been included in those tabulated. An open pitcher of *Darlingtonia californica* received 30 cc. of milk; 10 days later, the pitcher was characterized by a brown discoloration and shrunken areas indicative of serious injury. A similar condition has been noted in the field in pitchers of the Sarracenias which contained a very unusual bulk of insect captures.

Beef broth.—Raw lean beef was cut into fine strips, covered with water,

and permitted to simmer for one hour. The resulting broth was strained and cooled. The broth was used in stimulation experiments; the technic was exactly the same as when milk was used.

In all the experiments on *Darlingtonia californica*, the period of stimulation was 5 days. Each of 4 closed pitchers received 5 cc. of the broth; the stimulation produced an increase above the normal average volume of the liquid contents per pitcher of 387 percent. Each of 5 plugged pitchers received 10 cc. of broth; the resulting increase above the normal liquid contents was 308 percent. Fifteen cc. of the broth were introduced into an open pitcher; an increase above the normal liquid contents of 346 percent resulted.

In each of the experiments on *Sarracenia flava*, 9 pitchers were used; 10 cc. of broth were introduced into each pitcher; and the period of stimulation was 4 days. Closed pitchers showed an average increase above the normal liquid contents of 1787 percent, and a maximum increase of 2445 percent. Open pitchers showed an average increase of 683 percent, and a maximum increase of 1060 percent.

Meat.—Both raw beef and beef cooked by boiling were used in separate series of experiments on *Darlingtonia californica*. From 2 to 8 cubes (length of edge one-eight inch) of meat were dropped into each pitcher; the period of stimulation ranged between 5 and 7 days; details concerning the preparation of the pitchers and the measurement of the liquid contents have been given under the experiments with milk. An increase in the average volume of the pitcher contents was not noted when *cooked* beef was introduced into either plugged or open pitchers. The increase observed after the introduction of *raw* beef ranged from 48 percent in one lot of closed pitchers to 157 in one lot of open pitchers; intermediate values were noted in other pitchers of all three types.

The failure of the meat, raw or cooked, to produce a large increase in the volume of pitcher liquor, such as was produced by milk or by beef broth, is probably due to the fact that the solid meat exerted any stimulating action only on the small area of the pitcher lining with which it was in contact. The liquid foods occupied a larger volume and, therefore stimulated the far larger area of the pitcher lining with which they were in intimate contact. The bulk of the meat introduced did not equal that of the insect captures of many open pitchers; and an increase of the liquor to a volume far greater than that present in open pitchers was not to be expected.

Other foods of animal origin.—Experiments, similar to those with meat, were made on *Darlingtonia californica*, using raw egg white, cubes of the white of hard-boiled eggs, cheddar cheese, casein (Hammarsten), and fibrin. No definite response to the introduction of these substances was observed, probably for the same reasons as with meat.

Inorganic salts.—The following salts were used in aqueous solution: ferrous sulphate, ferric ammonium sulphate plus sodium acetate, potassium ferrocyanide, and potassium ferricyanide.

Five cc. of a 2 percent solution of ferrous sulphate were introduced into several pitchers of each of the following species: *Sarracenia Sledgei*, *S. flava*, and *S. Drummondii*. The pitchers were not injured at the end of several days, and an increase occurred in the volume of the pitcher liquor; in pitchers of *S. flava*, the liquor had increased to 4 times its normal volume.

A solution was prepared containing 2 grams of ferric ammonium sulphate (iron ammonium alum) and 2 grams of sodium acetate in each 100 cc.; the sodium acetate was added for its buffer effect, *i. e.*, to lessen the acidity otherwise imparted to the solution by the alum. This solution was introduced into pitchers of the three species mentioned above, 5 cc. to each pitcher. Several days later, the pitchers were obviously dying.

Potassium ferrocyanide and potassium ferricyanide were used in separate series of experiments on both closed and open pitchers of *Sarracenia flava*. Five cc. of a dilute solution of one of these salts were introduced into each pitcher; 4 days later the volume of the liquid contents of the pitcher was measured. Both salts produced an abundant secretion of pitcher liquor; this phenomenon was more marked in the case of potassium ferrocyanide. The increased secretion in one closed pitcher was almost 21 times the average normal secretion.

Response to Introduced Acid and Alkali

In the experiments on stimulation with milk, the pitcher contents frequently became acid to litmus. In order to ascertain whether the observed increase in the volume of the pitcher secretion was due to milk *per se* or to the acidity developed, the following studies were made with dilute acids. The response to the acids led to similar experiments with dilute alkali. The technic was that used in the experiments with milk. Except in two experiments, the reagent was permitted to remain in the pitcher for 5 days; in the experiments with single pitchers of *Sarracenia Sledgei* reported in Table IV, the acid re-

mained in the pitchers for 8 days. The reagents used were hydrochloric acid (symbol HCl, either 0.05 or 0.2 percent aqueous solution), acetic acid (abbreviated AcOH, either 0.05 or 0.1 percent aqueous solution), and sodium hydroxide (symbol NaOH, either 0.02 or 0.05 percent aqueous solution).

The following conclusions are deduced from a study of the data reported in Table IV:

TABLE IV.—RESPONSE OF THE PITCHERS OF CERTAIN *SARRACENIACEÆ* TO INTRODUCED ACID AND ALKALI

Genus and species.	*Number of pitchers.*	*Type of pitcher.*	*Reagent.*	*Volume of reagent introduced into each pitcher, cc.*	*Average volume of pitcher contents at end of experiment, cc.*	*Reaction of pitcher contents to litmus at end of experiment.*
Darlingtonia californica	15	Plugged	0.2% HCl	3.0	6.00	Neutral
Darlingtonia californica	6	Open	0.05% HCl	3.0	5.40	Neutral
Darlingtonia californica	1	"	0.05% HCl	10.0	11.00	Neutral
Darlingtonia californica	7	"	0.2% HCl	3.0	5.65	Neutral
Darlingtonia californica	6	"	0.2% HCl	10.0	*	Acid
Darlingtonia californica	2	"	0.2% HCl	30.0	*	Acid
Sarracenia Sledgei	2	"	0.05% HCl	5.0	2.20	†
" "	1	"	0.05% HCl	5.0	<0.50	Neutral
" *flava*	1	Closed	0.05% HCl	5.0	3.60	Faintly acid
" "	2	Open	0.05% HCl	5.0	5.00	Faintly acid
" *Drummondii*	5	"	0.05% HCl	5.0	3.55	Neutral
Darlingtonia californica	17	Plugged	0.1% AcOH	3.0	6.15	Neutral
Darlingtonia californica	1	Open	0.1% AcOH	3.0	6.00	Neutral
Sarracenia Sledgei	2	"	0.05% AcOH	5.0	2.10	Neutral
" "	1	"	0.05% AcOH	5.0	<0.50	Neutral
" *flava*	1	Closed	0.05% AcOH	5.0	2.80	Neutral
" "	3	Open	0.05% AcOH	5.0	2.67	Acid
" *Drummondii*	4	"	0.05% AcOH	5.0	1.20	Neutral
Darlingtonia californica	15	Plugged	0.02% NaOH	3.0	6.07	Neutral
Darlingtonia californica	4	Open	0.02% NaOH	3.0	6.80	Neutral
Sarracenia Sledgei	2	"	0.05% NaOH	5.0	4.50	Neutral
" *flava*	1	Closed	0.05% NaOH	5.0	4.00	Neutral
" "	1	Open	0.05% NaOH	5.0	2.40	Neutral
" *Drummondii*	3	"	0.05% NaOH	5.0	3.60	Neutral

Darlingtonia californica showed no marked trend to either increase or decrease the volume of its pitcher contents as a result of the introduction of dilute acid or dilute alkali.

* Pitchers injured, large areas brown and shrivelled.

† Contents of one pitcher faintly acid, of other pitcher neutral.

Almost invariably the volume of the liquid withdrawn from the pitchers of the Sarracenias was less than the volume of the introduced reagent. This shrinkage in volume was even greater than that shown by a comparison of the volume of reagent introduced with the average volume of the pitcher contents at end of experiment as given in Table IV, for the normal volume of pitcher liquor, which has already been reported in Table I, has not been included in Table IV. Therefore, evidence of absorption in the genus *Sarracenia* is unmistakable.

The reaction of the withdrawn pitcher contents was determined by means of litmus paper. The reaction, recorded in Table IV, should be compared with the reaction of the liquor of similar pitchers of the same species as reported in Table II. The pitcher contents of *Darlingtonia californica* returned to their normal neutral reaction within 5 days after the introduction of either dilute acid or dilute alkali. The contents of open pitchers of both *Sarracenia Sledgei* and *S. Drummondii* finally returned to their normal neutral reaction after introduction of any one of the three reagents. *Sarracenia flava* likewise showed a marked tendency for a return of its pitcher contents to their normal reaction within a few days after the introduction of either dilute acid or dilute alkali. This return of the pitcher contents to their normal reaction recalls the behavior of the human stomach under somewhat similar conditions as shown by the researches of Spencer, Meyer, Rehfuss, and Hawk.[70]

Action of the Pitcher Liquor of the Sarraceniaceæ upon Living Insects

Even prior to the opening of the pitcher, the pitcher liquor of certain species possesses the property of quickly rendering helpless those insects which come into contact with it. Reference has already been made to the work of Mellichamp[4,16] and Watson,[17] who studied the action of the pitcher liquor of *Sarracenia variolaris* (*S. minor*) upon insects. In the present research similar studies have been made, using the pitcher liquor of four other species of the *Sarraceniaceæ*. Unless otherwise stated, the pitcher liquor was collected on the same day that its action on insects was tested.

A typical experiment may be described. A location was chosen adjacent to a populous crater-shaped nest of a large brown ant abundant at Summerville, South Carolina. Wide mouth vials were used as containers. Pitcher liquor from *Sarracenia flava* was introduced into 10 vials, 2 cc. to the vial; 5

vials received liquor from closed pitchers and 5 vials liquor from open pitchers. Water was placed in another set of 10 vials, 2 cc. to the vial, in order to serve as a control experiment. The vials were of uniform size, and were arranged in suitable order for convenient observation. Worker ants were picked from the nest gently with the fingers; one ant was dropped into each vial, and the time recorded. Note was made of the time at which each ant ceased its struggles to escape, assumed a rigid position, and became incapable of motion even on jarring the vial. The elapsed time between the introduction of the ants and the cessation of their struggles ranged from 0.5 to 3.0 minutes with an average of 1.4 minutes in the experiments with liquor from closed pitchers, and from 1.5 to 2.0 minutes with an average of 1.6 minutes in the experiments with liquor from open pitchers. In the control experiments with water, 5 of the 10 ants ceased motion, the elapsed time ranging from 3.0 to 9.5 minutes, while the other 5 ants were still active at the end of 30 minutes.

Liquor from open pitchers of *Sarracenia flava* was collected at De Funiak Springs, Florida, in May. It was kept at room temperature without addition of a preservative, and was tested at Wilmington, Delaware, 49 days later, using the above technic. Large black ants of the genus *Campanotus* served as the experimental animals, 6 ants in the experiment proper and 6 ants in the control experiment. The average elapsed time to cessation of motion was 20 *seconds* in the experiment proper. In the control experiment, 3 of the ants ceased motion in from 5.25 to 7.45 *minutes*, while the other 3 ants were still active at the end of 46 *minutes*.

Experiments were made with the pitcher liquor of *Sarracenia Sledgei* at Mobile, Alabama, using large black ants of the genus *Campanotus*. The average elapsed time to cessation of motion was 48 seconds when 5 ants were introduced into liquor from open pitchers. Five ants were used in the control experiments, 1 ceased motion at the end of 180 seconds, the others were still active at the end of 300 seconds.

Experiments were also made on liquor from open pitchers of *Sarracenia Drummondii* at De Funiak Springs, Florida, using large brown ants. Five ants were introduced into the pitcher liquor and ceased motion in from 20 to 30 seconds; 5 ants which were introduced into distilled water, retained their motility for many minutes, and were finally permitted to escape at the conclusion of the experiment.

Typical experiments with the pitcher liquor from *Sarracenia Sledgei*,

S. flava, and *S. Drummondii* have been reported above. Similar results, concerning the action of the pitcher liquor on insects, have been obtained in other experiments—not reported in detail—on liquor from closed pitchers and from open pitchers of these species. The action of the pitcher liquor of *Darlingtonia californica* upon insects is described in a subsequent section.

Death of the ants was not coincident with their cessation of motion. Thus in the second experiment reported above (that made on black ants at Wilmington, Delaware), 6 ants ceased motion, on the average 20 seconds after their introduction into the pitcher liquor. Fifteen minutes after motion had ceased, they were removed from the pitcher liquor and placed on bibulous paper. They showed evidences of motion, on the average, 6 minutes, 22 seconds later, and were able to run away, on the average, 9 minutes after their removal from the liquor. The ants from other experiments behaved in a similar manner. However, permanent cessation of motion of the captured insects must inevitably lead to their death within the pitchers.

Insects sank in the pitcher liquor of the Sarracenias more frequently and more promptly than in water in the control experiments. This phenomenon suggested that the pitcher liquor has a surface tension less than that of water, and acts upon the insects by virtue of this property. Chemical, physical, and physiological tests were made in this connection.

Chemical, Physical, and Physiological Examination of the Pitcher Liquor

These tests were made with pitcher liquor to which no bactericide had been added.

Test for saponin.—Since saponins lower the surface tension of water when dissolved in that solvent, the hemolysis test for saponins was applied to samples of the pitcher liquor in order to ascertain if compounds of that group were present. The finger was pricked and blood was permitted to fall into 0.5 cc. of a 0.1 percent solution of sodium citrate in physiological (0.85 percent) solution of sodium chloride, until the volume of the latter had doubled. Equal volumes of the resulting suspension of human erythrocytes and the pitcher liquor were mixed on a glass slide, covered with a cover slip, and then viewed through a microscope. The erythrocytes remained intact at the end of 30 minutes; therefore, hemolysis did not occur, and saponins were not present in the pitcher liquor. This test was applied to samples of liquor from closed pitchers of *Darlingtonia californica*, *Sarracenia Sledgei*, *S. flava*, and *S. Drum-*

mondii, and from open pitchers of *S. flava* and *S. purpurea*. With all 6 samples, the test for a saponin was invariably negative.

The *physical tests* were both qualitative and quantitative. An oiled needle was used for a qualitative measurement of the surface tension. A needle 1.25 inches in length was oiled with olive oil. It readily floated on the surface of water for its entire length, and was not readily sunken by jarring the sides of the containing vessel. The oiled needle was also placed on the surface of the liquor from open pitchers of *Sarracenia flava* which was used in the experiments made on ants at Wilmington, Delaware, and reported above. The needle was floated on the surface of the pitcher liquor with difficulty. The needle then floated with both ends submerged, and sank very readily when the sides of the containing vessel were gently jarred. The same oiled needle was transferred, by means of dissecting forceps, 20 times in succession from the water to the pitcher liquor, then back to the water. *Throughout the entire series of transfers*, the needle was readily floated on the water and was not sunken in that medium by a heavy jarring of the table; on the other hand, the needle was floated on the pitcher liquor with difficulty and readily sank in the liquor on the slightest jarring of the table.

Quantitative measurement of the surface tension of the liquor from closed pitchers of *Sarracenia flava* was made by means of the du Noüy surface tension apparatus,[71] as manufactured by the Central Scientific Company.[72] The measurements were made at a temperature of 25° C. The pitcher liquor was found to have a surface tension of 66.4 dynes per cm., while a sample of distilled water, tested at the same time, was found to have a surface tension of 75.0 dynes per cm. Hence the surface tension of the pitcher liquor was considerably lower than that of water.

Physiological tests.—The action of liquor from closed pitchers of *Sarracenia flava* was tested upon frogs and a guinea-pig.

The pitcher liquor was injected into the anterior lymph sac of *frogs;* 5 animals were used; the dose per gram of body weight ranged from 0.0035 to 0.05 cc., and the actual dose from 0.12 to 0.49 cc. The frogs were observed at intervals for 9 hours, and remained alive and active. The next day, two of the frogs were pithed. In both of them, the lymph sac showed no evidence of irritation; however, it was practically free from mucus which is normally present. Possibly the mucus had been digested by the proteolytic enzyme of the pitcher liquor.

One cc. of the pitcher liquor was injected subcutaneously into a *guinea-pig* weighing 527 grams; no evil effects were noted. Three days later, 3 cc. of the same sample of pitcher liquor were administered subcutaneously to the same guinea-pig without the production of any evil effects. Five days after the second injection the guinea-pig showed some adhesions in the region of the injections but was otherwise uninjured.

Action of the Pitcher Liquor of *Darlingtonia californica* Upon Living Insects

The action of the liquor from both closed and open pitchers of *Darlingtonia californica* was tested on ants, locusts (short-horned grasshoppers, family *Acrididæ*), and bumble bees (genus *Bombus*). These experiments were made in Plumas County, California.

In one experiment, 25 vials were prepared, and 3 cc. of liquor from closed pitchers were introduced into each vial. For controls, 3 cc. of water were introduced into each of another group of 25 vials. A large ant was dropped into each vial. The number of ants still active and struggling to escape was:– At the end of 1 minute, pitcher liquor 18, water 17; at the end of 5 minutes, pitcher liquor 11, water 8; at the end of 10 minutes, pitcher liquor 8, water 8; at the end of 30 minutes, pitcher liquor, 6, water 8. These results showed practically no difference between the pitcher liquor and the water with respect to their action on ants.

Similar experiments were made with bumble bees and with locusts, using liquor from closed and from open pitchers separately; the period of observation extended over several hours in each experiment. The results showed that the action of the pitcher liquor on these insects with respect to motility did not differ materially from that of water.

None of the insects—ants, bees, or locusts—sank in the pitcher liquor more frequently or more promptly than in water.

THE ENZYMES OF THE PITCHER LIQUOR OF THE SARRACENIACEÆ

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PROTEASE

A series of experiments on the occurrence of a protease in the pitcher liquor of *Sarracenia flava* was described at the general meeting of the American Philosophical Society in 1917.[18] These experiments have been continued and extended to include the other North American representatives of the family. Each species has been studied separately. Tests have been made on liquor from closed pitchers and on filtered liquor from open pitchers. In the field experiments, carmine fibrin was used as the substrate. This substrate and others were used in laboratory experiments. As a rule, the test for the presence of a protease by means of carmine fibrin was made in an acid medium containing 0.2 percent hydrochloric acid, also in an alkaline medium containing 0.5 percent sodium carbonate; frequently it was also carried out without the addition of either acid or alkali; in every case, trikresol (0.2 percent) was used as a bactericide. Two reagents were prepared; one contained 2.2 percent hydrochloric acid and 2.2 percent trikresol; the other contained 5.5 percent sodium carbonate and 2.2 percent trikresol; by addition of 1 volume of a reagent to 10 volumes of pitcher liquor, the proper degree of acidity or alkalinity was imparted to the reaction mixture. Control or blank tests were carried out, using pitcher liquor which had been boiled, then cooled to the temperature of the atmosphere prior to addition of the reagents. The temperature of digestion was that of the atmosphere. Each separate test was usually made in duplicate, however, at times, in triplicate or even in quadruplicate. Unless otherwise stated, each test was made on a composite sample of liquor drawn from a number of pitchers.

With four species—*Darlingtonia californica, Sarracenia Sledgei, S. flava,* and *S. Drummondii*—edestan and casein were used as substrates in laboratory tests for the presence of a protease in the pitcher liquor. In these tests, the temperature of digestion was 37.5° C. The pitcher liquor had been preserved by addition on one-tenth its volume of a 2.2 percent aqueous solution of

trikresol; therefore 1.1 cc. of preserved liquor equalled 1.0 cc. of liquor as drawn from the pitchers.

In the experiments with *edestan*, a solution of that substrate was prepared by solution of 0.1 gram of edestin in 100 cc. of 0.1 percent hydrochloric acid by heating to boiling, then cooling to room temperature. In each experiment 1.1 cc. of preserved pitcher liquor were mixed with 2.0 cc. of edestan solution. After incubation for 1.5 hours, 0.5 cc. of a saturated aqueous solution of sodium chloride was added to the reaction mixture. If digestion of the substrate had not occurred, a cloudy precipitate of edestan formed; if partial digestion had occurred, a faint cloud formed; if the edestan had been completely digested, the solution remained perfectly clear. The controls in these experiments always yielded a cloudy precipitate.

In the experiments with *casein*, a freshly prepared solution of that substrate was used. A beaker, containing 0.1 gram of purified casein (caseinogen), 5 cc. of 0.1 normal sodium hydroxide solution and 25 cc. of distilled water, was placed on a wire gauze and heated until boiling occurred and the casein dissolved. The beaker and its contents were quickly cooled to room temperature. The solution was rendered neutral by addition of 0.1 normal hydrochloric acid, and was diluted to a total volume of 100 cc. In each experiment, 1.1 cc. of preserved pitcher liquor were mixed with 2 cc. of the substrate solution. The mixture was incubated at a temperature of 37.5° C., usually for 2 hours. Then 6 drops of a solution of acetic acid in dilute alcohol (1 part by volume of glacial acetic acid, 49 parts of distilled water and 50 parts of 95 percent alcohol) were added. If digestion of the substrate had not occurred, a cloudy precipitate of casein formed; if partial digestion had occurred, a faint cloud formed; if complete digestion had occurred the solution remained perfectly clear. The controls always yielded a cloudy precipitate. Whenever the pitcher liquor was so acid in reaction that a precipitate immediately formed on mixing it with the substrate solution, then the experiment was repeated using pitcher liquor which had previously been rendered neutral to litmus by addition of an aqueous solution of sodium carbonate.

Coagulated egg white was used as the substrate in certain experiments on the liquor from closed pitchers of *Sarracenia flava*, *S. Drummondii* and *S. Sledgei* and from open pitchers of *S. flava*. A cube of white of hard-boiled egg, 0.25 inch to the edge, was placed in 5 cc. of pitcher liquor; 0.2 percent of trikresol was used as a bactericide. This substrate was also used in the presence of acid and of alkali.

All experiments in which either carmine fibrin or egg white served as the substrate, were made in vials which were tightly closed with cork stoppers in order to prevent evaporation of their contents.

The action of the pitcher liquor upon the various substrates is described by species in the following paragraphs.

SARRACENIA FLAVA

The experiments on *Sarracenia flava*, reported in Table V, demonstrate that a proteolytic enzyme, acting on carmine fibrin, is present in the liquor of closed pitchers as well as in that of open pitchers. They further show that this enzyme is far more active in the presence of 0.2 percent hydrochloric acid than in the presence of 0.5 percent sodium carbonate.

TABLE V.—DIGESTION OF CARMINE FIBRIN BY PITCHER LIQUOR OF *SARRACENIA FLAVA*

Type of pitchers.	*Reaction of medium.*	*Volume of pitcher liquor, cc.*	*Mass of carmine fibrin, gram.*	*Solution of substrate.*	
				Marked in hours.	*Complete in hours.*
Closed	0.2% HCl	10	0.20	3	12
		5	0.20	3	15
		5	0.20	3	26
		5	0.20	2.5	17
		5	0.20	<16	<27
		3	0.05	3.5	<14
		3	0.05	8	<14
		4	0.05	7	<14
		3	0.05	5	..
Open	0.2% HCl	10	0.20	2	8
		4	0.05	..	15
		3	0.05	48	96
Closed	0.5% Na_2CO_3	3	0.05	67	76
		4	0.05	..	*
Open	0.5% Na_2CO_3	4	0.05	144	†

The proteolytic enzyme had practically no action on carmine fibrin in the absence of added acid or alkali. In four separate experiments, from 3 to 10 cc. of liquor from *closed* pitchers were permitted to act upon carmine fibrin. In three separate experiments, from 4 to 10 cc. of liquor from *open* pitchers were permitted to act upon that substrate. In each experiment, either 0.05 or 0.20 gram of carmine fibrin was used, with 0.2 percent trikresol as a bactericide. The period of observation varied with the experiment, and ranged from 5 to 42 days. The substrate was not appreciably attacked in any of the seven experiments.

* Solution incomplete at end of 8 days.
† Solution incomplete at end of 40 days.

The edestan test, previously described, was applied to four samples of liquor from closed pitchers and to two samples of liquor from open pitchers. The substrate was completely digested in 1.5 hours by all six samples. A third sample of liquor from open pitchers showed almost complete digestion of the substrate when the period of digestion was extended to 2 hours. In an experiment on liquor from closed pitchers, the substrate was almost all digested on incubation at room temperature for 30 minutes, and was completely digested on incubation at the usual temperature (37.5° C.) for the same period of time.

The casein test, previously described, was also used. On digestion for 2 hours, the casein was partially digested by three of the four samples of liquor from closed pitchers, and by both samples of liquor from open pitchers.

In the experiments with coagulated egg white, four series of tests were made. In two series, only trikresol was added, and incubation occurred at temperatures of 49° to 52° C. and 37.5° C., respectively. In the third series sufficient hydrochloric acid was added to give a concentration of 0.2 percent of that acid, in the fourth series sufficient sodium carbonate to give a concentration of 0.5 percent of that salt; trikresol was used in both series, and incubation occurred in them at a temperature of 37.5° C. In these series, use was made of one composite sample of liquor from each type of pitcher, closed and open. The substrate was not attacked in any of the four series by liquor from either type of pitcher on digestion for a period of 120 hours.

Sarracenia Drummondii and Sarracenia Sledgei

The liquor from both closed pitchers and open pitchers of *Sarracenia Drummondii* and of *Sarracenia Sledgei* contains a protease which digests carmine fibrin in the presence of 0.5 percent sodium carbonate, as may be seen on reference to Table VI. Tests were also made to ascertain the activity of this enzyme in the presence of dilute acid, and also without the addition of either acid or alkali. Each experiment was made on a separate gathering of pitcher liquor.

Six experiments were made on liquor from *closed* pitchers of *S. Drummondii* in the presence of 0.2 percent hydrochloric acid, using 0.05 gram of carmine fibrin and from 2.0 to 4.2 cc. of liquor in each experiment. The period of observation ranged from 12 to 54 days. Four experiments were carried out with liquor from *closed* pitchers of *S. Sledgei* in the presence of 0.2 percent hydrochloric acid; the mass of carmine fibrin ranged from 0.025 to 0.05 gram, the

volume of liquor from 2.0 to 3.8 cc., the period of observation from 12 to 60 days. The substrate was not even partially dissolved in any of these ten experiments.

TABLE VI.—DIGESTION OF CARMINE FIBRIN BY PITCHER LIQUOR OF *SARRACENIA DRUMMONDII* AND *SARRACENIA SLEDGEI* IN AN ALKALINE MEDIUM

Species and Type of Pitcher	*Volume of Pitcher Liquor, cc.*	*Mass of Carmine Fibrin, gram.*	*Concentration of Sodium Carbonate, percent.*	*Solution of Substrate Complete in hours.*
Sarracenia Drummondii Closed	1.9	0.05	0.5	2.5
	3.0	0.01	0.5	3.5
	3.0	0.05	0.5	4.5
	2.0	0.05	0.5	<18
	2.0	0.05	0.5	<18
Sarracenia Drummondii Open	3.5	0.025	0.5	1.5
	3.0	0.01	0.5	<14
	3.0	0.05	0.5	<14
	2.0	0.05	0.5	<18
Sarracenia Sledgei Closed	3.5	0.025	0.5	1.5
	1.6	0.01	0.5	3.5
	2.0	0.05	0.5	<18
	2.0	0.05	0.5	<18
Sarracenia Sledgei Open	3.5	0.05	0.5	<14
	3.5	0.025	0.5	1.5
	2.0	0.05	0.5	5.5
Sarracenia Drummondii Open	3.0	0.01	0.5	<11
	3.0	0.01	0.25	<11
	3.0	0.01	0.125	<11
	3.0	0.01	0.0625	<11
	3.0	0.01	0.03125	36
Sarracenia Drummondii Closed	3.0	0.01	0.5	3.5
	3.0	0.01	0.25	5 days
	3.0	0.01	0.125	32 days *
	3.0	0.01	0.0625	†
	3.0	0.01	0.03125	†

In the experiments with liquor from *open* pitchers of both species in the presence of 0.2 percent hydrochloric acid, the volume of pitcher liquor used ranged from 2.0 to 3.5 cc., the mass of carmine fibrin from 0.025 to 0.05 gram. Four experiments were made with liquor of *Sarracenia Drummondii*, the period of observation ranged from 12 to 57 days; the substrate was not attacked in three of the experiments; in the fourth experiment it was partially dissolved at the end of 31 days and practically completely dissolved at the end of 49 days. Three experiments were made with liquor of *Sarracenia Sledgei*, the period of observation ranging from 5 to 57 days; the carmine fibrin was not attacked in two experiments; in the third experiment it was partially dissolved in 37 days and practically completely dissolved in 57 days.

In those experiments which were made on the pitcher liquor without the addition of either acid or alkali, 0.2 percent trikresol was used as a bactericide,

* Partial solution.
† No solution at end of 50 days.

as in all the other experiments. Six experiments were carried out with liquor from *closed* pitchers of *Sarracenia Drummondii*, using from 1.9 to 3.0 cc. of liquor and 0.05 gram of carmine fibrin. In four experiments, which were under observation for periods of 50 to 55 days, the substrate was not attacked. In one experiment the carmine fibrin was completely dissolved in 7 days; in another experiment it showed marked solution at the end of 21 days. Two experiments were made with liquor from *closed* pitchers of *Sarracenia Sledgei*, using 2.0 cc. of liquor and 0.05 gram of substrate; in one experiment complete solution of the carmine fibrin occurred in 7 days, in the other experiment, marked solution in 21 days.

Four experiments were carried out with liquor from *open* pitchers of *Sarracenia Drummondii*; the volume of liquor ranged from 2.0 to 3.5 cc., the mass of carmine fibrin from 0.01 to 0.05 gram. In one experiment, solution of the substrate had not occurred at the end of 21 days; in the other three experiments, solution slowly occurred, being noticeable at the end of approximately 30 days and practically complete at the end of approximately 50 days.

Three experiments were conducted with liquor from *open* pitchers of *Sarracenia Sledgei*, using from 1.5 to 3.5 cc. of liquor and from 0.01 to 0.05 gram of carmine fibrin. In one experiment, solution of the carmine fibrin had not occurred in 9 days. In the other experiments, the substrate was practically completely dissolved in 37 days and 49 days respectively.

In these experiments, as in all the other experiments, digestion of the substrate did not occur in the controls.

The experiments with carmine fibrin demonstrate that the pitcher liquor of *Sarracenia Drummondii* and *S. Sledgei* contains a protease and that this enzyme acts best in an alkaline medium. The liquor from closed pitchers did not show proteolytic activity in the presence of 0.2 percent hydrochloric acid; and that from open pitchers showed this activity only occasionally and on prolonged digestion. As recorded in a preceding section, the liquor from closed pitchers of these species is distinctly acid to litmus. Probably the native acid plus the hydrochloric acid produced a degree of acidity at which the enzyme was inactive. When neither acid nor alkali was added to the pitcher liquor, the substrate, at times, was dissolved on prolonged digestion.

Since the protease acted best in an alkaline environment, experiments were made in which the concentration of sodium carbonate was varied, ranging from 0.5 to 0.03125 percent, while the mass of carmine fibrin and the volume

of pitcher liquor remained constant. The liquor was obtained from pitchers of *Sarracenia Drummondii*. The results are recorded in the bottom section of Table VI. With liquor from open pitchers, the substrate was completely dissolved during the night, in less than 11 hours, at all concentrations of sodium carbonate from 0.5 to 0.0625 percent, and was completely dissolved in 36 hours when the sodium carbonate had a concentration of 0.03125 percent. With liquor from closed pitchers, the time required for solution of the carmine fibrin increased as the concentration of the sodium carbonate decreased. In fact with the higher dilutions of that salt, even partial solution had not occurred at the end of 50 days. In this connection it should be noted that the acid present in the liquor from closed pitchers exerted a neutralizing effect on the sodium carbonate; the reaction mixtures containing 0.0625 and 0.03125 percent of that salt were respectively neutral and acid to litmus, while the others in the series were alkaline to litmus.

The edestan test and the casein test were applied to two samples of liquor from closed pitchers of *Sarracenia Drummondii*, two samples from closed pitchers of *S. Sledgei*, and one sample from open pitchers of *S. Drummondii*.

In the edestan test, after incubation for 1.5 hours, no digestion of the substrate had been produced by the liquor from closed pitchers, while a slight digestion had been produced by that from open pitchers. The edestan, which had been precipitated by the sodium chloride, was not further digested when incubation was continued for an additional period of 70.5 hours at a temperature of 37.5° C.

In the casein test, that protein was completely digested by all five samples of pitcher liquor on incubation for 2 hours at a temperature of 37.5° C. This test was also applied to a third sample of liquor from closed pitchers of *Sarracenia Drummondii;* the casein was completely digested in 8.5 hours.

Coagulated egg white was also used as a substrate with liquor from *closed* pitchers of both species. Two samples of liquor from *Sarracenia Drummondii* and one sample from *S. Sledgei* were permitted to act on egg white in the presence of trikresol without the addition of either acid or alkali. The action of each sample was studied at two temperatures, 37.5° C. and 49° to 52° C. The substrate was not attacked in any of these tests during a period of 120 hours. Other experiments were made in which the pitcher liquor was permitted to act upon the egg white at a temperature of 37.5° C. in the presence of either dilute acid or dilute alkali. The liquor from *S. Sledgei* had no action

on the substrate on incubation for 120 hours in the presence of 0.2 percent hydrochloric acid. In the presence of 0.5 percent sodium carbonate, it produced marked digestion of the egg white in 24 hours; solution was almost complete in 72 hours and complete in 120 hours. Both samples of liquor from *S. Drummondii* had a proteolytic action on the egg white in the presence of 0.5 percent sodium carbonate; incipient digestion was noted in 24 hours, marked digestion in 48 and 144 hours, and advanced digestion in 168 and 216 hours respectively.

Sarracenia rubra

Opportunity did not occur to procure abundant material for study of the pitcher liquor of *Sarracenia rubra*. Experiments were made with a single sample of liquor from closed pitchers, using 1.3 cc. of liquor and 0.01 gram of carmine fibrin in each experiment. In the presence of 0.5 percent sodium carbonate and trikresol, the substrate was completely dissolved in 2 hours. In the presence of 0.2 percent hydrochloric acid and trikresol, partial solution was noted at the end of 9 days, complete solution at the end of 50 days. These results indicate that the pitcher liquor of this species contains a protease which acts best in an alkaline medium.

Sarracenia minor

Field tests were made on one sample of liquor from closed pitchers and on one sample from open pitchers of *Sarracenia minor*. The action of each sample was studied on carmine fibrin in the presence of (1) 0.2 percent trikresol, (2) trikresol plus 0.2 percent hydrochloric acid, and (3) trikresol plus 0.5 percent sodium carbonate. Neither sample exerted any proteolytic action on the substrate on digestion for 30 days in the presence of trikresol without the addition of either acid or alkali. In the case of the *closed* pitchers, 1.6 cc. of liquor and 0.01 gram of carmine fibrin were used in each test; the substrate was partially dissolved in 6 hours and completely dissolved in 15 hours in the presence of 0.2 percent hydrochloric acid; it was almost completely dissolved in 9 days in the presence of 0.5 percent sodium carbonate. In the case of the *open* pitchers, 3.0 cc. of liquor and 0.05 gram of carmine fibrin were used in each test; the substrate was partially dissolved in 6 hours and completely dissolved in 15 hours in the presence of 0.2 percent hydrochloric acid; it was partially dissolved in 15 hours and almost completely dissolved in 27 hours in the presence of 0.5 percent sodium carbonate.

These results indicate that the pitcher liquor of *Sarracenia minor* (*S. variolaris*) contains a protease which acts best in an acid environment, although it also acts, though less rapidly, in an alkaline medium.

Sarracenia psittacina

Sarracenia psittacina was found in Walton County, Florida, in early May, in some abundance but not of maximum size; the new pitchers for the year were just open or about to open. Although these pitchers were obviously secreting liquor in small amounts, yet it was insufficient in quantity to be collected by means of a fine pipette. An attempt was made to secure a diluted pitcher liquor by introduction of approximately 0.5 cc. of water into each of 50 open pitchers which were free from captures; four hours later, the water was collected by means of a pipette, and the composite sample was used in tests for a protease. Four cc. of the collected water and 0.01 gram of carmine fibrin were used in each test. The reaction mixtures were kept under observation for a period of 4 months. No evidence of digestion was obtained in the presence of 0.2 percent trikresol, or in the presence of that bactericide plus 0.5 percent sodium carbonate, or in the controls. In the presence of trikresol plus 0.2 percent hydrochloric acid, the substrate, which had swollen in the usual manner, gradually decreased in volume, but had not dissolved at the end of 4 months. These results indicate that *Sarracenia psittacina* may possibly secrete into its pitcher cavity a protease which is active in the presence of 0.2 percent hydrochloric acid. However, conclusive experiments with this species must be deferred until it is found growing vigorously in an accessible locality.

Sarracenia purpurea

With respect to the amount of liquor present in its closed pitchers, *Sarracenia purpurea* presents difficulties almost as great as *S. psittacina*. However, a pitcher of *S. purpurea* is occasionally found in which the secretion, usually present as a fine, perspiration-like beading on the inner wall of the pitcher, has collected into drops at its bottom.

Two experiments were made in which open pitchers of living plants were thoroughly flushed by a current of water under pressure, then emptied as completely as possible. From 10 to 15 cc. of water were introduced into each pitcher. After the lapse of a number of days, the water was removed by means of a pipette; and the composite sample was subjected to tests for a

protease, in the presence of acid and of alkali, and without the addition of either reagent.

In one experiment two pitchers were used, and the water remained in them for 15 days. In each test 6 cc. of the collected water was permitted to act upon 0.01 gram of carmine fibrin. The collected water almost completely digested the substrate in 57 days in the presence of 0.5 percent sodium carbonate and trikresol. In the other experiment seven pitchers were used, and the water remained in them for 10 days. Fourteen cc. of the collected water were permitted to act upon 0.20 gram of carmine fibrin in each test. The collected water produced marked digestion of the substrate in 40 hours, and completely dissolved it in 64 hours in the presence of 0.5 percent sodium carbonate and trikresol. The substrate was not dissolved in either experiment within the times stated, in the presence of 0.2 percent hydrochloric acid and trikresol, or in the presence of that bactericide without the addition of either acid or alkali.

Liquor from open pitchers was used in a series of digestion experiments in which carmine fibrin served as the substrate. On reference to Table VII it is seen that the liquor contained a protease which completely dissolved the substrate in a short period of time in the presence of 0.5 percent sodium carbonate and trikresol.

The experiment on May 23 was made on liquor freshly collected from open pitchers which had matured during the preceding season and remained green through the winter. The remaining experiments were made on material from pitchers maturing during the season in which the liquor was collected. On November 15 the liquid contents of the pitchers were frozen to cores of ice which were thawed when the samples were collected; therefore the enzyme had not been destroyed by freezing. These two experiments (May 23 and November 15) indicate the retention of proteolytic activity by the liquor throughout the life of the pitcher.

The digestion experiments made in the presence of 0.2 percent hydrochloric acid and trikresol (Table VII) show that the protease may be slightly active under these conditions.

The experiments of June 30, July 1, and July 14 also were carried out in the presence of 0.2 percent trikresol without the addition of either acid or alkali. The period of observation ranged from 2 to 61 days; the substrate was not even partially dissolved in any of these experiments. All of the ex-

periments on this species thus far described were made on material gathered from strong vigorous plants in Ocean County, New Jersey. Liquor was also collected from smaller and less vigorous open pitchers growing in Tolland County, Connecticut, late in July. Ten cc. of this liquor failed to even partially dissolve 0.01 gram of carmine fibrin in 36 days in the presence of 0.5 percent sodium carbonate and trikresol, or 0.2 percent hydrochloric acid and trikresol, or 0.2 percent trikresol without the addition of either acid or alkali.

TABLE VII.—DIGESTION OF CARMINE FIBRIN BY LIQUOR FROM OPEN PITCHERS OF *SARRACENIA PURPUREA*

Reaction of medium.	*Date of collection.*	*Volume of pitcher liquor, cc.*	*Mass of carmine fibrin, gram.*	*Solution of substrate* Marked in	Complete in
0.5% Na_2CO_3..	May 23	25	0.20	..	87 hours
	June 5	2	0.01	72 hours	120 "
	June 30	10	0.05	8 "	48 "
	July 1	3	0.01	24 "	48 "
	July 14	25	0.20	..	42 "
	July 16	8	0.01	..	48 "
	November 15	25	0.20	87 "	135 "
0.2% HCl.....	June 5	2	0.01	42 days	..
	June 30	10	0.05	..	*
	July 1	3	0.01	..	†
	July 14	25	0.20	..	‡
	July 16	8	0.01	47 days	..

Darlingtonia Californica

Study was made of the action of liquor from closed, plugged, and open pitchers of *Darlingtonia californica* on various substrates. Carmine fibrin and fibrin were used most frequently. From 5 to 15 cc. of pitcher liquor and from 0.01 to 0.20 gram of one of these substrates were used in each experiment, with 0.2 percent trikresol as a bactericide. Some tests were made in the presence of 0.2 percent hydrochloric acid, others in the presence of 0.5 percent sodium carbonate, still others without the addition of either acid or alkali. The period of observation was usually 10 days, frequently 30 days, occasionally 40 days. Field experiments were made at atmospheric temperature, laboratory experiments at a temperature of 37.5° C. A total of 57 such experiments were performed—19 with fibrin and 38 with carmine fibrin; no digestion of the substrate was produced by liquor from any of the three types of pitcher.

In the edestan test, the period of digestion was prolonged, ranging from 4 to 7 days. Digestion of the edestan was not produced by liquor from closed pitchers (2 samples), plugged pitchers (2 samples), and open pitchers (1

* No solution in 16 days.

† No solution in 61 days.

‡ No solution in 48 hours.

sample). Two other samples of liquor from open pitchers produced a very slight digestion of the edestan.

In the casein test, the period of digestion was prolonged, ranging from 1 to 4 days. No digestion of the substrate was produced by liquor from closed pitchers (1 sample) and plugged pitchers (2 samples). The casein was completely digested by two samples of liquor from open pitchers.

In two field experiments, solid casein was used as a substrate. Several milligrams of casein and 5 cc. of pitcher liquor were permitted to digest in the presence of 0.2 percent hydrochloric acid and trikresol. One experiment was made with liquor from closed pitchers, the other with that from open pitchers. The casein was not dissolved at the end of 18 days.

In another series of field experiments, approximately 0.25 gram of solid casein was suspended in 3 cc. of water and introduced into a closed pitcher which was then plugged in the manner already described. Ten pitchers were treated in this manner. When the pitchers were opened 5 days later, undissolved casein was found at the bottom of each pitcher.

The protean derived from castor bean globulin was also used as a substrate in one experiment on liquor from closed pitchers and in one experiment on that from open pitchers. In each experiment, 2 cc. of a 2 percent solution of the globulin in a 5 percent aqueous solution of sodium chloride were mixed with 2 cc. of pitcher liquor and 0.5 cc. of 0.1 normal hydrochloric acid; and sufficient trikresol was added to make the concentration of that bactericide 0.2 percent. The period of incubation was 6 days at a temperature of 37.5° C. The protean, which was precipitated on addition of the acid, was not even partially dissolved in either experiment.

In certain field experiments, coagulated egg white, raw egg white, raw beef, and cooked beef were introduced into pitchers; and study was made of the action of the pitcher liquor upon them.

Four cubes of white of hard-boiled egg—one-eighth inch to the edge—were introduced into each of ten vigorous pitchers which had just opened. Seven of these pitchers were cut open for examination at the end of 72 hours, the remainder at the end of 144 hours. The cubes were found submerged in the pitcher liquor, unchanged in appearance, and with sharp edges. This procedure was repeated with eight closed pitchers and with eight plugged pitchers. The cubes were removed from the pitcher liquor at the end of 24 hours, and found unaltered.

Raw egg white was diluted with water in the ratio of 1 to 9, and was introduced into ten mature plugged pitchers, 10 cc. of the dilution into each pitcher. Five days later the contents of the pitchers were withdrawn; the liquid was somewhat glairy, and yielded a voluminous precipitate on boiling.

Cubes of raw fresh lean beef—one-eighth inch to the edge—were introduced into eight closed, fourteen plugged, and four open pitchers. The pitchers were cut open from 5 to 7 days later; the cubes had not undergone any disintegration, having retained their shape and size. This entire experiment was repeated with cubes of cooked beef with the same result.

The weight of the evidence, comprised in all the foregoing digestion experiments, is that a protease, secreted by the plant, does not occur in the pitcher liquor of *Darlingtonia californica*. The only evidence of the presence of a protease was the slight digestion of edestan and the complete digestion of casein, both produced by certain samples of liquor from open pitchers in laboratory experiments in which extremely small amounts of substrate were used while the period of incubation was prolonged; it is quite possible that this digestion was produced by enzymes of bacterial origin. For none of the tests for the detection of a protease revealed the presence of such an enzyme in the liquor from either closed or plugged pitchers; and, with the exceptions just noted, none of the tests showed the presence of such an enzyme in the liquor from open pitchers.

Stability of the Protease at Room Temperature

A composite sample of liquor from closed pitchers of *Sarracenia flava* was divided into two portions: one portion was preserved by addition of sufficient trikresol to produce a concentration of 0.2 percent of that bactericide; both portions were then kept at the temperature of the room, and tested at intervals with respect to their action on carmine fibrin in the presence of 0.2 percent hydrochloric acid and trikresol. In each test, use was made of 0.05 gram of substrate. The requisite control tests were also made. The data have been collected in Table VIII. The initial test was made on the day of collection.

The results obtained with the liquor preserved with trikresol demonstrate conclusively that the protease in the pitcher liquor retained its activity for over a year under the conditions stated. It is of interest to note that the liquor, to which no bactericide had been added, also exhibited proteolytic

activity during a like period. These results are in harmony with the occurrence of a protease in the liquor obtained in the spring (May 23) from open pitchers of *Sarracenia purpurea* which had matured during the preceding season and remained green throughout the winter.

TABLE VIII.—DIGESTION OF CARMINE FIBRIN BY PITCHER LIQUOR OF *SARRACENIA FLAVA* KEPT AT ROOM TEMPERATURE

Pitcher Liquor	*Period of keeping, days.*	*Volume of Pitcher Liquor, cc.*	*Solution of Substrate*	
			Marked in hours.	*Complete in hours.*
Preserved with trikresol	0	4	7	<18
	18	3	15	24
	34	3	..	21
	54	3	24	<120
	67	3	7	<23
	370	3	21	46
Without bactericide	0	4	7	<18
	18	3	24	40
	34	3	..	21
	54	3	24	<120
	67	3	7	<23
	370	3	..	21

Retention of Proteolytic Activity After Dilution of the Pitcher Liquor

It is a matter of common observation that beating rains enter the pitchers of most species of *Sarracenia*, necessarily diluting their normal liquid contents. As is well known, enzymes continue to act on dilution of their solutions. The following experiments were therefore undertaken to ascertain if the protease of the pitcher liquor obeys this general law, and continues to act on dilution of the liquor, a phenomenon which necessarily occurs in nature.

The liquor was collected from pitchers and diluted with sterile water in varying proportions. These dilutions were permitted to act on carmine fibrin in the presence of 0.2 percent trikresol. In each experiment, the reaction (acid or alkaline) was that which had been found most suitable for the protease of that particular species in the experiments already described.

The results, which have been collected in Table IX, demonstrate that the protease of the pitcher liquor retained its activity on dilution of the latter; appreciable masses of carmine fibrin were completely dissolved in a few hours even when the degree of dilution was as great as 1:17.

Stimulation Experiments

Separate studies were made of the influence of mechanical stimulation and of food stimulation upon the proteolytic activity of the pitcher liquor. The technic of stimulation has been described in the preceding paper.

TABLE IX.—DIGESTION OF CARMINE FIBRIN BY DILUTED PITCHER LIQUOR

Species of Sarracenia	*Type of Pitcher.*	*Degree of Dilution of Pitcher Liquor.*	*Volume of Diluted Pitcher Liquor, cc.*	*Reaction.*	*Mass of Substrate, gram.*	*Solution of Substrate. Marked in hours.*	*Solution of Substrate. Complete in hours.*
S. Drummondii	Closed	1:2	3	0.5% Na_2CO_3	0.01	..	3
		1:3	3	"	0.01	..	4
		1:5	3	"	0.01	..	4.5
		1:9	3	"	0.01	..	<18
		1:17	3	"	0.01	..	<18
S. Drummondii	Open	1:3	3	"	0.01	..	<10
		1:5	3	"	0.01	..	<10
		1:9	3	"	0.01	..	<10
S. Drummondii	Open	3:10	10	0.125% Na_2CO_3	0.05	..	<10
		1:10	10	"	0.05	10	<35
S. flava	Closed	1:8	8	0.2% HCl	0.05	6	<18
S. flava	Open	1:8	8	0.2% HCl	0.05	15	48

Mechanical stimulation did not cause the secretion of a protease by *Darlingtonia californica*. With respect to proteolytic activity, liquor of mechanically stimulated pitchers of *Sarracenia Sledgei*, *S. flava*, and *S. Drummondii* did not differ from liquor of unstimulated pitchers of these species.

Attention has already been called to the remarkable response of the pitchers of *Darlingtonia californica* to stimulation by certain *foods* and *reagents*. As a result of the stimulation, the secretion of pitcher liquor was greatly increased. However, a protease was not present in the pitcher liquor of this species after stimulation by the following substances: Sterile milk, raw egg white, coagulated egg white, raw beef, boiled beef, meat broth, fibrin, and acetic acid, hydrochloric acid, and sodium hydroxide in the concentrations stated in the preceding paper.

Summary of Protease Studies

The weight of the evidence is that a protease, secreted by the plant, does not occur in the pitcher liquor of *Darlingtonia californica*.

Sarracenia psittacina possibly secretes a protease active in the presence of dilute acid.

The other species of *Sarracenia* unquestionably secrete proteases. As is shown in one of the following papers, the liquor in closed pitchers is bacteriologically sterile, yet it contains a protease; therefore that enzyme is secreted by the pitcher. Proteolytic activity is exhibited by liquor from both closed pitchers and open pitchers.

The proteases of *Sarracenia flava* and *S. minor* act best in the presence of dilute acid, but also exhibit some activity in the presence of dilute alkali.

The proteases of *S. Sledgei*, *S. Drummondii*, *S. rubra*, and *S. purpurea* act best in the presence of dilute alkali, but also exhibit some activity in the presence of dilute acid. In our experiments, the dilute acid was 0.2 percent hydrochloric acid, the dilute alkali 0.5 percent sodium carbonate. The proteases of *S. Sledgei* and *S. Drummondii* also exhibit some activity without the addition of either acid or alkali.

As is shown in the preceding and following papers, the hydrogen-ion concentration of the liquor from individual pitchers almost always lies on either the acid or the alkaline side of true neutrality. This fact, and the more or less marked activity of the proteases in either an acid or an alkaline environment would indicate that the proteases are active in the liquor in the pitchers, and have a part in the digestion of the prey prior to absorption.

Neither mechanical nor food stimulation of the pitchers increased the proteolytic activity of their liquor.

The protease was active after dilution of the pitcher liquor, thereby duplicating conditions which occur in the native habitat of the Sarracenias.

When the pitcher liquor of *Sarracenia flava* was kept at room temperature, its protease retained its activity for a period of more than a year. The liquor in pitchers of *Sarracenia purpurea* exhibited proteolytic activity during the entire life of the pitchers.

It is of interest to note that proteases have been found in the pitcher liquor of genera of all three families of pitcher plants: *Sarraceniaceæ*, *Cephalotaceæ and Nepenthaceæ*. The work of previous investigators and of ourselves on the *Sarraceniaceæ* is described in this monograph. In previous publications, we have reviewed the work of other investigators on *Nepenthes*, the only genus of the *Nepenthaceæ*, [64, 65] and have described our work on the occurrence of a protease in the pitcher liquor of this genus.[66] A protease, active in the presence of dilute hydrochloric acid, has recently been found by Dakin[67] in the pitcher liquor of *Cephalotus follicularis*, the only genus and species of the *Cephalotaceæ*.

Other Enzymes

Tests for the presence of enzymes, other than proteolytic, were made on composite samples of liquor from pitchers of *Darlingtonia californica* and of *Sarracenia flava*. The pitcher liquor was collected in the field and was mixed with one-tenth its volume of 2.2 percent aqueous solution of trikresol. The sample was shipped to Philadelphia and was examined on its arrival.

The two genera were studied in different years; and all the conditions were not exactly the same in the two series of tests. However, in the tests for urease, diastase, and the inverting enzymes (maltase, emulsin and invertase or sucrase), the ratio of the volume of the pitcher liquor to the concentration of the substrate was kept constant by use of 1.0 cc. of a 1 percent aqueous solution of the substrate for each 1.1 cc. of the sample of pitcher liquor containing trikresol which actually represented 1.0 cc. of the liquor as collected. The temperature of incubation was always 37.5° C. The requisite blank or control experiments were always made using pitcher liquor which had been boiled, then cooled to the temperature of the atmosphere. Whenever necessary, after the pitcher liquor and the substrate had been mixed, sufficient 2.2 percent solution of trikresol was added to bring the concentration of that bactericide in the reaction mixture to 0.2 percent. Urea was used as the substrate for urease, alpha methyl *d*-glucoside for maltase, amygdalin for emulsin, sucrose (cane sugar) for invertase, and soluble starch for diastase. All these reagents were used as 1 percent aqueous solutions prepared by solution of a weighed amount of the substrate in the requisite volume of distilled water at room temperature. Tributyrin was used as the substrate for lipase and ethyl butyrate for esterase. In the tests for diastase and the inverting enzymes, the volume of the composite sample of pitcher liquor used was:—*Darlingtonia californica* 11.00 cc., *Sarracenia flava* 2.75 cc.; these volumes corresponded to 10.00cc. and 2.50 cc. respectively of the liquor as actually collected.

Composite samples of the liquor from closed pitchers of *Darlingtonia californica* and from open pitchers of that species were tested separately for the presence of urease, maltase, emulsin, invertase, and diastase. A composite sample of the liquor from closed pitchers of *Sarracenia flava* was tested for the presence of these five enzymes and of lipase and esterase.

Inverting enzymes.—After incubation for 4 days, both the determination proper and the control were tested for the presence of reducing sugar by means of Benedict's qualitative alkaline copper solution. Neither the determination proper nor the control reduced Benedict's solution in the tests for maltase, emulsin, and invertase in the liquor from closed pitchers of *Darlingtonia californica*, or in the tests for maltase and emulsin in the liquor from open pitchers of that species, or in the tests for maltase and emulsin in the liquor from closed pitchers of *Sarracenia flava*. The determination proper reduced Benedict's solution in the test for invertase in the liquor from open

pitchers of *Darlingtonia californica*, and in the test for that enzyme in the liquor from closed pitchers of *Sarracenia flava*, while the corresponding controls had absolutely no reducing action. The test for invertase was repeated on a second composite sample of liquor from open pitchers of *Darlingtonia californica;* the period of incubation was 12 days; the result was the same as that obtained with the first sample.

These results indicate that the closed pitchers of *Darlingtonia californica* do not contain any of the three enzymes:—maltase, emulsin, or invertase. The liquor in open pitchers of this species does not contain either maltase or emulsin but may contain invertase; the invertase may be of bacterial origin since not present in the liquor of closed pitchers. The liquor in closed pitchers of *Sarracenia flava* does not contain either maltase or emulsin, but does contain invertase.

Diastase.—The period of incubation was as long as 12 days. At intervals, aliquots of both the determination proper and its control were tested for the presence of reducing sugar by means of Benedict's qualitative alkaline copper solution, and for the color reaction with iodine dissolved in an aqueous solution of potassium iodide.

The experiments with the first composite sample of pitcher liquor from closed pitchers of *Darlingtonia californica* were indecisive. After incubation for 10 days, both the determination proper and its control reduced Benedict's solution, and both yielded a blue color with iodine. The test was also applied to a second composite sample of liquor from closed pitchers of this species, the period of incubation being 12 days. The determination proper produced a faint reduction of Benedict's solution, its control no reduction; both gave a blue color with iodine.

With the first composite sample of liquor from open pitchers of *Darlingtonia californica*, after incubation for 4 days, the determination proper gave a slight reduction of Benedict's solution, its control no reduction; after incubation for 10 days, the determination proper gave the reaction of erythrodextrin (red color) with iodine, while its control still gave the blue color produced by starch. A second composite sample of liquor from open pitchers of this species was also tested; the period of incubation was 12 days. The determination proper markedly reduced Benedict's solution, and gave no color with iodine; its control had no action on Benedict's solution and yielded a blue color with iodine.

When the composite sample of liquor from closed pitchers of *Sarracenia flava* was subjected to this test, the result was exactly the same after incubation for 4 days and for 10 days. Neither the determination proper nor its control reduced Benedict's solution, and both yielded a blue color with iodine.

These results indicate that a trace of diastase may be present in the liquor of closed pitchers of *Darlingtonia californica*. Liquor from open pitchers of this species possesses a distinct diastasic activity, but it may be, at least in part, of bacterial origin. The liquor from closed pitchers of *Sarracenia flava* does not contain a diastase.

Urease.—In the tests for the presence of urease, use was made of 11.00 cc. of a composite sample of pitcher liquor from *Darlingtonia californica* or of 5.50 cc. of that from *Sarracenia flava*. These samples had been diluted slightly by addition of trikresol in the field; and the volumes used represented 10.00 cc. and 5.00 cc. respectively of actual pitcher liquor. After the pitcher liquor and the solution of the substrate had been mixed and the proper amount of trikresol solution added, the resulting solution was rendered neutral to methyl orange. After incubation, the solutions were alkaline, and were titrated with 0.1 normal hydrochloric acid using methyl orange as an indicator in order to measure quantitatively the produced alkali.

In the experiments with *Darlingtonia californica*, the period of incubation was 3 days. In each of the four titrations—the determination proper with liquor from closed pitchers and its control and the determination proper with liquor from open pitchers and its control—0.25 cc. of 0.1 normal hydrochloric acid was required to neutralize the alkali.

In the experiment with liquor from closed pitchers of *Sarracenia flava*, both the determination proper and its control required 0.50 cc. of 0.1 normal hydrochloric acid to neutralize the alkali produced during incubation for 4 days.

Since the alkalinity of the determination proper did not exceed that of its control in any of the tests, urease was not present in any of the samples of pitcher liquor examined.

Lipase and *Esterase.*—In testing for the presence of these enzymes in the liquor from closed pitchers of *Sarracenia flava*, 1 cc. of the substrate (tributyrin for lipase, ethyl butyrate for esterase) was mixed with 5.50 cc. of the composite sample of pitcher liquor as collected. The mixture was rendered neutral to phenolphthalein, and was then incubated for 4 days. The liberated butyric

acid was then titrated with 0.1 normal sodium hydroxide using phenolphthalein as an indicator.

In the test for esterase, both the determination proper and its control required 0.10 cc. of 0.1 normal sodium hydroxide in the final titration, therefore esterase was not present.

In the test for lipase, in the final titration with 0.1 normal sodium hydroxide, the determination proper required 0.50 cc. and its control only 0.40 cc. Therefore lipase was probably present.

Summary.—The preceding results lead to the following general conclusions:

The liquor from closed pitchers of *Darlingtonia californica* contained a trace of diastase, while maltase, emulsin, invertase, and urease were absent.

The liquor from open pitchers of *Darlingtonia californica* contained invertase and diastase while maltase, emulsin, and urease were absent. However, no invertase and only a trace of diastase occurred in the liquor from closed pitchers; and these enzymes may have been formed in the open pitchers by bacteria which are present in the contents of open pitchers but are absent from the liquor in closed pitchers.

The liquor from closed pitchers of *Sarracenia flava* contained invertase and probably lipase, while maltase, emulsin, diastase, urease, and esterase were absent.

CHEMICAL CONSTITUENTS OF THE PITCHER LIQUOR OF THE SARRACENIACEÆ

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Hydrion Concentration of the Pitcher Liquor

The hydrion concentration of the pitcher liquor was determined in the field by the colorimetric method devised by Wherry [73, 74, 75, 76, 77, 78, 79] for the study of soil reactions. Dr. Wherry had made certain determinations on the liquor of mature pitchers of *Sarracenia purpurea* prior to the inclusion of this phase of the biochemistry of the *Sarraceniaceæ* within the scope of our research. All values, whether determined by him or by ourselves, are expressed as *specific reactions, i.e., specific acidity* or *specific alkalinity.* We are indebted to him for the following results on liquor from open pitchers of various species of *Sarracenia.* Each test, unless otherwise stated, was made on liquor from a single pitcher.

Sarracenia Purpurea

May 8, 1919. Essick Heights, Pa., Bog water acid 300; pitcher liquor acid 300.

May 13, 1919. Green Pond, N. J. Swamp water alkaline 30 to acid 30; spagnum hummocks in which plants grew acid 300; pitcher liquor 5 tests, 1 alkaline 3, 1 neutral, 3 acid 3 to 30.

May 14, 1919. Dover, N. J. Swamp water acid 300; pitcher liquor 3 tests, 3 acid 30 to 300.

June 20, 1919. West Burke, Vt. Bog water acid 300; pitcher liquor acid 300.

August 7, 1919. Nuangola, Pa. Bog water acid 300; pitcher liquor acid 300.

January 1, 1921. Gary, Ind. Bog water alkaline 3; muck at base of plants neutral; pitcher liquor acid 3.

June 28, 1922. Wilmington, N. C. Sandy soil acid 300; pitcher liquor acid 300.

August 20, 1922. Laurel, Md. Swamp soil acid 300; pitcher liquor acid 300.

August 3 to 18, 1923. Mount Desert Island, Me. New Mill Meadow. Stream water acid 3, peat soil acid 30, liquor from young pitcher alkaline 10, liquor from older pitcher of same plant acid 300. Witch Hole. Lake water acid 30, sphagnum soil acid 1,000,

liquor from young, just opened pitcher acid 1,000, old pitcher alkaline 10.

Great Cranberry Island. Peat soil acid 1,000, pitcher liquor 10 tests, acid 100 to 3,000.

September 9, 1923. Mineral Springs, Ind. Swamp water alkaline 3 to 10, soil hummocks neutral to acid 300, pitcher liquor several tests acid 10 to 1,000.

Other Species

S. minor. June 20, 1922. Cox, Ga. Mud soil acid 30, pitcher liquor acid 300. Peat soil acid 300, pitcher liquor acid 300.

June 24, 1922. Summerville, S. C. Woodland soil acid 300, pitcher liquor acid 300.

June 25, 1923. Buffton, S. C. Pitcher liquor acid 1,000.

S. flava. June 25, 1921. Flat Rock, N. C. Sandy meadow soil acid 300; pitcher liquor acid 300.

June 24, 1922. Summerville, S. C. Soil acid 300; pitcher liquor 6 tests, 6 acid 30 to 300.

June 29, 1922. Winter Park, N. C. Peaty meadow soil acid 300; pitcher liquor acid 300.

June 25, 1923. Near Statesville, Ga. Pitcher liquor acid 3,000.

S. rubra. June 29, 1922. Winter Park, N. C. Sandy soil acid 300; the pitchers contained no liquor, distilled water was introduced into them and attained a reaction of acid 300.

June 25, 1923. Near Statesville, Ga. The pitchers contained no liquor; distilled water, used as in the preceding experiment, attained a reaction of acid 300.

S. psittacina. June 25, 1923. Near Statesville, Ga. The pitchers contained no liquor; distilled water was introduced into them and attained a reaction of acid 300.

Our tests on *Sarracenia purpurea*, made at Whitings and Davenport, Ocean County, N. J., showed the following range in reaction. Unless otherwise stated, the pitcher liquor was obtained from large, well developed, open pitchers containing much liquor, and each test was made on liquor from a single pitcher.

November 15, 1919. Bog water acid 300; pitcher liquor 7 tests, 1 alkaline 3, 6 acid 3 to 300.

May 23, 1920. Bog water acid 300; pitcher liquor 10 tests, 10 acid 300 to 1,000.

May 29, 1920. Bog water acid 300; pitcher liquor 5 tests, 5 acid 10 to 1,000.

July 7, 1920. Bog water acid 300; pitcher liquor 6 tests, 2 alkaline 3, 4 acid 10 to 1,000.

September 15, 1920. Bog water acid 300; pitcher liquor 8 tests, 8 acid 300 to 1,000.

The tests in May were made on pitchers of the preceding season, the new pitchers not having yet opened.

Three additional tests were made using newly opened pitchers containing few captures and a small volume of liquor. In all three pitchers, the liquor was alkaline 3 in reaction. A composite sample of liquor from 50 such pitchers was also tested, and found to be neutral (alkaline 1, acid 1). These additional tests seem to indicate that the alkaline reaction is more prevalent in the liquor of newly opened active pitchers, while the reaction is more frequently acid in the liquor of older pitchers.

In *our work* on the southern species, tests were made on the liquor from open pitchers of *Sarracenia Sledgei*, *S. flava*, and *S. Drummondii*. The experiments on *S. Sledgei* were made at Biloxi, Miss., May 10, 1921. Using individual pitchers, the reaction of the liquor was neutral in 2 pitchers, alkaline 3 in 1 pitcher, and ranged from acid 3 to acid 10 in 2 pitchers. A composite sample of liquor from 40 pitchers was neutral in reaction. At the time these tests were made, the volume of liquor in each pitcher was very small, and the liquor was diluted with neutral distilled water in the experiments on individual pitchers.

The experiments on *S. flava* were made at De Funiak Springs, Florida, May 19, 1921. The liquor from 5 pitchers, tested separately, was invariably acid; the reaction ranged from acid 100 to acid 3,000.

The experiments on *S. Drummondii* were made at Freeport, Florida, May 22, 1921. Five individual pitchers were tested; the liquor in 3 pitchers had a reaction of acid 3, that in 2 pitchers a reaction of alkaline 3.

Constituents of the Pitcher Liquor

The *nitrogen content* of the liquor has been determined for various types of pitchers of *Darlingtonia californica* and *Sarracenia flava*. Liquor from

open pitchers was filtered prior to analysis. The determination was made by the Gunning modification of the Kjeldahl method. The sample was mixed in a Kjeldahl flask with 10 grams potassium sulphate, a tiny fragment of cupric sulphate, and 25 cc. concentrated sulphuric acid. Heat was applied until the water had been driven off; and the determination was then carried out in the usual manner. The samples were preserved for analysis by addition of trikresol immediately after their collection in the field. The results are reported in Table X.

TABLE X.—TOTAL NITROGEN CONTENT OF PITCHER LIQUOR

Sample Number	*Genus and Species.*	*Type of Pitcher*	*Actual Volume of Pitcher Liquor in Sample cc.*	*Weight of Nitrogen in Sample mg.*	*Weight of Nitrogen in 1 cc. of Pitcher Liquor, mg.*
1.	*Darlingtonia californica*	Closed	45.45	1.26	0.027
2.	*Darlingtonia californica*	Plugged	22.75	0.35	0.015
3.	*Darlingtonia californica*	Plugged	45.45	0.42	0.009
4.	*Darlingtonia californica*	Open	45.45	1.54	0.034
5.	*Darlingtonia californica*	Open	45.45	2.24	0.049
6.	*Sarracenia flava*	Closed	25.00	0.91	0.036
7.	*Sarracenia flava*	Open	50.00	3.01	0.060
8.	*Sarracenia flava*	Open	25.00	1.26	0.050

Gross Composition.—A composite sample of liquor from closed pitchers of *Darlingtonia californica* had a specific gravity of 1.003 at 15° C. It contained 0.213% total solids, 0.104% ash, and 0.046% calcium oxide (lime). The lime formed 44.23% of the ash. The total solids were determined by drying a known mass (approximately 15 grams) of the pitcher liquor, free from preservatives, in a platinum dish at the temperature of boiling water until the weight became constant. The ash was determined by incinerating the total solids in a muffle furnace at a dull red heat. The calcium was precipitated as the oxalate and weighed as the sulphate. Chlorides were present in the liquor, for it yielded a white curdy precipitate with an aqueous solution of silver nitrate; this precipitate dissolved in ammonia water, and was reprecipitated from the resulting solution by nitric acid.

Tests for Reducing Sugar.—Composite samples of liquor from closed pitchers of *Darlingtonia californica* and *Sarracenia Sledgei*, and from open pitchers of *D. californica*, *S. Sledgei* and *S. Drummondii*—5 samples in all were tested with respect to their action on Benedict qualitative alkaline copper solution. This reagent was not reduced by any of the samples; therefore, reducing sugar was not present in the pitcher liquor examined. It is, however, possible that the liquor may at times be contaminated by sugar derived from the nectar.

Presence of Hydrogen Sulphide in the Pitcher Liquor under Certain Conditions

In certain experiments (page 60) on *Darlingtonia californica*, cubes of coagulated egg white were introduced into open pitchers, and examined 144 hours later. The contents of one of the pitchers then had an odor of hydrogen sulphide.

In other experiments on this species, raw egg white was diluted with ninefold its volume of water and introduced into 10 plugged pitchers. Five days later, the contents of these pitchers were collected. A composite sample of the contents was placed in a test tube, acidified with hydrochloric acid, and heated. A piece of filter paper, which had been moistened with an aqueous solution of lead acetate, was held at the mouth of the tube. The evolved gas blackened the paper. Therefore, hydrogen sulphide was present in the contents of these pitchers.

A BACTERIOLOGICAL STUDY OF THE PITCHER LIQUOR OF THE SARRACENIACEAE

By JOSEPH SAMUEL HEPBURN, A.M., B.S. in Chem., M.S., Ph.D. and ELISABETH QUINTARD ST. JOHN, M.D.

Bacteriological studies have been made of the liquor from both closed pitchers and open pitchers of plants growing in their native habitat. Up to date, all the North American *Sarraceniaceae* except *Sarracenia psittacina* have been drawn into the scope of these studies. The liquor from each pitcher was studied separately. The procedure for the collection and laboratory examination of the pitcher liquor was an amplification of that in our study of *Nepenthes*.[68]

Closed Pitchers

Technic. The external surface of the pitcher, near the base of the cavity and slightly above the level of the pitcher liquor, was sterilized by passage through the flame of an alcohol lamp. The pitcher was then cut obliquely through the sterilized region by means of sterile scissors. The upper portion of the pitcher was discarded. The lower portion contained the pitcher liquor, which was immediately poured into a tube containing a "slant" of sterile plain nutrient agar. The usual precautions were observed in making this transfer, *i. e.*, to sterilize the scissors in the flame just before use, to flame the top of the tube and its cotton plug just before removal of the plug to make the transfer, and again before insertion of the plug after making the transfer. In making the transfer by pouring, the pitcher liquor came into contact only with a small area of the inner wall of the pitcher. The tubes were immediately sent to Philadelphia by express.

In a few instances, a sterile platinum loop was used to transfer the pitcher liquor to a tube of sterile gelatin. These tubes were brought to Philadelphia by one of us.

Since closed pitchers of *Sarracenia purpurea* contain so very little liquor, the technic was modified somewhat for this species.

The top of the closed pitcher was passed through the flame of an alcohol lamp. The top was then removed by means of sterile scissors. One cc. of sterile water was immediately introduced into the pitcher cavity by means of a sterile pipette. By imparting a gentle motion to the pitcher, this water

was made to wash the inner wall of the cavity. The water was then transferred, by use of another sterile pipette, to the agar slant.

Upon arrival at Philadelphia, the tubes were incubated for four days at a temperature of 37° C.

A total of 50 experiments were made, each on liquor from a single closed pitcher. These experiments were distributed as follows: *Darlingtonia californica* 3, *Sarracenia minor* 3, *S. Sledgei* 3, *S. flava* 15, *S. Drummondii* 3, *S. rubra* 3, *S. purpurea* 20.

Results. Neither colonies nor other evidence of proliferation of bacteria developed in any of the 50 experiments. Therefore, the pitcher cavity and liquor of closed pitchers is bacteriologically sterile.

Open Pitchers

Technic. The procedure for the transfer of liquor from an open pitcher to a slant of sterile agar was exactly the same as was followed in the case of closed pitchers. However, in the case of open pitchers of *Sarracenia purpurea*, 1 cc. of the liquor was transferred from the pitcher cavity to the agar slant by means of a sterile pipette. A separate pitcher containing prey was used for each experiment.

A total of 39 experiments were made, distributed as follows: *Darlingtonia californica* 3, *Sarracenia minor* 5, *S. Sledgei* 4, *S. flava* 8, *S. Drummondii* 3, *S. rubra* 4, *S. purpurea* 12.

In all 39 experiments, colonies developed upon the agar slants. Therefore, bacteria were always present in the liquor of open pitchers. Sterile physiological (0.85 percent) sodium chloride solution was added to each agar culture, and an emulsion of the bacteria was prepared. This emulsion was then used for the inoculation of the sterile media enumerated below. No effort was made to isolate bacterial species and to test their action in pure culture upon the various media, for the sole purpose of the bacteriological experiments was to permit the bacteria to act on the media in as nearly as possible the same manner as they act on the prey in the pitcher cavity.

The following media were used. The figure in parentheses after each medium shows the number of separate experiments in which it was employed.

I. Substrates for proteolytic bacteria.

A. Gelatin (38).

B. Nährstoff-Heyden agar (28).

C. Loeffler blood serum (35).
D. Dorset egg medium (32).
E. Aleuronat-protein agar (6).
F. Casein agar (6).
G. Fibrin agar (3).
H. Ovalbumin agar (3).
I. Litmus milk (28).

II. Substrates for alkali-forming bacteria.
A. Ammonium lactate rosolic acid agar (34).
B. Ammonium tartrate rosolic acid agar (31).
C. Acetamide rosolic acid agar (34).
D. Urea rosolic acid agar (31).
E. Asparagin rosolic acid agar (34).
F. Glycocoll rosolic acid agar (34).

III. Substrates for acid-forming bacteria.
A. Litmus lactose agar (19).
B. Litmus glucose agar (19).

IV. Substrates for the colon-aerogenes group.
A. Lactose bile salt bouillon (49).
B. Trypsinized peptone (28).

The following media are frequently used in routine bacteriological work, and were made in the usual manner: Litmus milk, litmus lactose agar, litmus glucose agar, lactose bile salt bouillon, Dorset egg medium, and Loeffler blood serum. The gelatin[82] and the trypsinized peptone[83] were prepared and used according to the directions of Rivas. The various protein agars and rosolic acid agars were made as suggested by Crabill and Reed.[84] They had as their base a medium containing magnesium and ferrous sulphates, dipotassium phosphate, potassium chloride, and agar. To this base was added either a protein or a simple nitrogenous organic compound, to serve as the sole source of nitrogen and carbon for the bacteria. The various protein agars were made by addition of 1 percent of a protein (Nährstoff-Heyden, aleuronat-protein, casein, fibrin, ovalbumin) to this plain agar prior to sterilization, and thorough suspension of the protein in the sterile mass prior to use. The various rosolic acid agars were made by additions of (*a*) one-half percent by volume of a 2 percent solution of rosolic acid in 60 percent alcohol and (*b*) a simple nitrogenous compound (ammonium lactate or tartrate, acetamide,

urea, asparagin, glycocoll) to the plain agar. One percent of asparagin was used, the other compounds in molecular concentration equal to that of the asparagin. The rosolic acid agars were always sterilized by the discontinuous method. The production of basic compounds (amines or ammonia) by bacteria growing on these media was shown by the red color imparted to the medium beneath and around the colony. Sterile plates of rosolic acid media were always poured, to serve as controls for the determination of the change in color in the experiment proper.

The media, after inoculation with the suspension of the bacteria, were incubated at a temperature of 37° C., and examined at intervals for evidences of bacterial action. Unless otherwise stated, observations were made during a period of 30 days.

Results. The results obtained with each medium may be summarized briefly.

Gelatin. The gelatin was completely liquefied in all 38 experiments. In approximately three-fourths of the experiments, liquefaction was complete by the end of the first week of incubation.

Nährstoff-Heyden agar. Colonies developed in 26 of the 28 experiments. Clearing of the cloudy medium about the colonies occurred in 7 of these experiments, and was apparent on some plates as early as the fifth day of incubation. This clearing of the medium was due to the action of proteolytic bacteria on the Nährstoff-Heyden, which, according to Gotschlich,[85] is a mixture of albumoses (proteoses).

Loeffler blood serum. Digestion of this medium occurred in four-fifths of the 35 experiments. The digestion was slight in 2 experiments and marked in 26 experiments; it became apparent as early as the fifth day of incubation, and tended to become more pronounced as the period of observation lengthened. A putrid odor was evolved in many of the experiments; it became apparent as early as the tenth day of incubation.

Dorset egg medium. Distinct digestion of the medium occurred in 15 experiments. It was noted as early as the fifth day of incubation, and tended to become more marked as the period of incubation increased. Incipient digestion of the medium was observed in 4 additional experiments. Therefore evidence of digestion was present in 59.4 percent of the 32 experiments. A putrid odor developed in many of the experiments, and became apparent as early as the tenth day of incubation.

Aleuronat-protein agar. While colonies developed in all 6 experiments, digestion of the protein with clearing of the medium about the colonies occurred in but 2 experiments.

Casein agar. The bacteria proliferated in all 6 experiments. Digestion of the casein with clearing of the medium about the colonies took place in 3 experiments.

Fibrin agar. Colonies developed in all 3 experiments, but the fibrin was not digested to any appreciable extent.

Ovalbumin agar. Growth of the bacteria occurred in all 3 experiments. However, digestion of the coagulated albumin did not occur to an appreciable extent.

Litmus milk. Coagulation of the milk, *i. e.*, the casein, occurred in all 28 experiments. The coagulum was then digested in 22 experiments; evidence of digestion became apparent as early as the fifth day of incubation; usually from fifty to eighty percent of the coagulum had been dissolved by the end of the period of observation. The litmus was bleached in three-fourths of the experiments.

Rosalic acid agars. The number of experiments in which each of these media was used has been recorded above (see page 77). The entire group of experiments may be summarized in tabular form, reporting for each substrate: (*a*), the percent of experiments in which colonies developed, and (*b*) the percent of experiments in which alkalinity was produced.

	Percent of experiments showing	
Substrate	*Growth*	*Alkalinity*
Ammonium lactate	91.2	88.3
Ammonium tartrate	96.8	83.9
Acetamide	91.2	91.2
Urea	77.4	52.3
Asparagin	97.1	97.1
Glycocoll	94.1	91.2

This alkalinity characterized the colonies and the medium about them, and frequently extended over the entire plate. It could be produced in any one of four ways:—

1. By oxidation of the organic nitrogenous compounds, including the ammonium salts, to ammonium carbonate.

2. By hydrolysis of an amide, *e. g.*, acetamide, and oxidation of the resulting ammonium salt to ammonium carbonate.

3. By desaminization of an amino acid with liberation of ammonia.

4. By decarboxylization of an amino acid with the formation of an amine.

Of the substrates used, acetamide and urea are amides, glycocoll is an amino acid, asparagin is both an amino acid and an amide, and ammonium lactate and tartrate are ammonium salts of organic acids.

A grand total of 198 plates of these rosolic acid agars were inoculated. In 22 percent of them an odor of ammonia or amines developed during the earlier portion of the period of incubation.

Toward the end of this period, a fading or bleaching of the rosalic acid occurred in approximately 40 percent of all the plates inoculated.

Litmus agars. The litmus lactose agar and the litmus glucose agar were used in the same 19 experiments. With the lactose medium, a permanent acidity developed in 2 experiments, a primary acidity followed by a secondary alkalinity in 1 experiment, and a permanent alkalinity in 16 experiments. With the glucose medium, a permanent acidity developed in 4 experiments, a primary acidity followed by a secondary alkalinity in 5 experiments, and a permanent alkalinity in 10 experiments.

These results indicate that the bacterial flora of the liquor in open pitchers attacked the peptone of the medium in preference to its carbohydrate. This was especially true of the lactose medium.

Lactose bile salt bouillon. In 38 experiments, the emulsion of bacteria was sown into lactose bile salt bouillon contained in Dunham fermentation tubes. In each of 11 additional experiments, a 2 cc. sample of liquor from an open pitcher was sown into a sterile tube of this medium in the field, using a sterile pipette to collect the liquor from the pitcher and transfer it to the tube. The inoculated tubes were carried to Philadelphia.

The period of incubation with this medium was 5 days at a temperature of 37° C. The production of gas and its collection in the inner inverted tube (positive reaction) was presumptive evidence of the presence of the colon-aerogenes group of bacteria, commonly called the *Bacillus coli* group.

Of the 49 experiments in which this medium was used, gas was produced in 26 experiments, or 53 percent of the total. The test was always negative (no gas-formation) with liquor from open pitchers of *Darlingtonia californica.* The test was also applied to liquor from open pitchers of each species of *Sarracenia* except *S. psittacina;* with each species, some pitchers yielded positive, some negative results, although they were growing near each other. These

results indicate that the *Bacillus coli* group, if present, is introduced by the captured insects and not from the bog water of the habitat. This conclusion is further supported by the experiments on *Sarracenia purpurea*. The 11 field experiments, mentioned above were made with this species. Two bogs lay on opposite sides of a railroad, and a stream of water flowed through both bogs. Of 7 samples of pitcher liquor, taken in one bog, 5 gave positive and 2 negative results. Of 4 samples, taken in the other bog, all yielded negative results. The liquor from 12 open pitchers growing in these bogs was also studied, using the agar slant technic. The bacterial emulsions thus obtained gave 9 positive and 3 negative tests for the presence of the *B. coli* group. Therefore, of 23 open pitchers in these bogs, 14 (61 percent) presumptively contained members of the colon-aerogenes group while 9 did not. Duplicate samples of water, each containing 2 cc., were taken from the stream, and from each bog in the immediate vicinity of the pitchers whose liquor was used in the field tests. Sterile pipettes were used to collect the water and transfer it to the sterile tubes of the medium. The samples of water and of pitcher liquor were taken at the same time. The stream water and the water of both bogs did not produce gas in the medium. Therefore the organisms of the *B. coli* group, which were present in 61 percent of the *Sarracenia purpurea* pitchers examined, did not come from the surrounding water, and must have been carried into the pitchers by the prey. The absence of this group of micro-organisms from the open pitchers of *Darlingtonia californica* was doubtless due to the very sparsely settled region in which that species grows, and the consequent failure of its prey to become infected by members of the group.

Trypsinized peptone. With this medium, the period of incubation was 5 days at a temperature of 37° C. The test for the presence of indol was then made by means of para-dimethylaminobenzaldehyde and hydrochloric acid. A purplish red color developed if indol had been produced in the medium by the bacteria. A positive reaction for indol was obtained in 21 experiments, *i. e.*, in 75 percent of the 28 experiments in which this medium was used.

Now in these 28 experiments, the lactose bile salt bouillon had also been employed, and gas had been produced in 15 experiments, i. e. in 53.6 percent.

Since indol formation occurred in a higher percent of these experiments than did gas formation, the conclusion may be drawn that indol-forming bacteria, other than members of the *B. coli* group, may be present in the liquor of open pitchers.

General Summary

Closed pitchers. The pitcher cavity and liquor of closed pitchers invariably was bacteriologically sterile.

Open pitchers. The liquor from open pitchers, which had captured prey, invariably contained bacteria. The bacterial flora of this liquor always included species which digest proteins. Gelatin was always liquefied. Loeffler blood serum and the casein of litmus milk were digested in approximately 80 percent of the experiments in which these respective media were used. The proteins of Dorset egg medium were attacked in about 60 percent of the experiments in which it was employed. The proteins in the various protein-agars were not markedly attacked, but supported growth of the bacteria. As a rule, the bacteria from a given open pitcher produced proteolysis of at least 2 or 3 substrates and frequently of 4 or 5 substrates.

However, the bacteria digested the proteins so slowly that their part in the digestion of the prey must be a minor one on the genus *Sarracenia*, the protease of the pitcher liquor playing the leading rôle. The bacteria apparently live in symbiosis with the Sarracenias, drawing their nutriment from the digested insects, and aiding, to a certain extent, in the digestion of the prey. Since the pitcher liquor of *Darlingtonia californica* does not contain a protease, the proteolytic bacteria, present in the open pitchers of this species, must be the chief factor in the digestion of the prey. Doubtless, in the pitchers of both genera (*Sarracenia* and *Darlingtonia*), tissue enzymes of the captured insects participate in the digestion by virtue of their autolytic action.

A putrid odor was frequently produced by the action of the bacteria on the Loeffler blood serum and the Dorset egg medium. This phenomenon recalls the observations concerning the presence of hydrogen sulphide in the pitcher contents of *Darlingtonia californica* several days after egg white had been introduced, see page 73.

The bacteria exhibited a marked tendency to attack protein (peptone) in preference to carbohydrate. This was shown by the production of an alkaline reaction in the litmus agars, especially litmus lactose agar. When the bacteria from a given pitcher produced an alkaline reaction in the litmus agars, they also produced an alkaline reaction in the rosalic acid agars.

The bacteria were able to use amino acids, amides, and the ammonium salts of organic acids as their sole source of nitrogen and carbon, and, as a result, render the reaction alkaline to rosolic acid; and, at times, they produced

an odor of ammonia and amines. As a rule, the bacteria from a given pitcher grew and produced an alkaline reaction in 5 or 6 of the rosolic acid media. An alkaline reaction to rosolic acid means a hydrogen-ion concentration, pH, numerically equal to or greater than 8.0,[86] or a specific alkalinity numerically equal to or greater than 10. However, the pitcher should be able to maintain the normal reaction of its liquor even if basic compounds be produced in its cavity, just as it maintains the normal reaction when dilute acid or dilute alkali is introduced into the cavity (see page 44).

Members of the colon-aërogenes group of bacteria were present in the liquor of over half of the open pitchers. They apparently were introduced into the pitcher cavity by the prey.

Indol-producing bacteria, not members of the colon-aërogenes group, also were frequently present in the liquor of open pitchers.

THE ABSORPTION OF NUTRIENTS IN THE PITCHERS OF THE SARRACENIACEÆ

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The technic used and the results obtained in this part of the research have been described in detail elsewhere.[19] They may be summarized briefly.

Study was made of:

1. The absorption of water from the pitcher cavity of *Darlingtonia californica*, *Sarracenia Sledgei*, *S. flava*, and *S. Drummondii*.

2. The absorption of nitrogenous compounds, such as ammonium chloride, ammonium tartrate, acetamide, urea, asparagin, glycocoll, trypsinized peptone, peptone, and egg albumin, from the pitcher cavity of *Sarracenia Sledgei*, *S. flava*, *S. Drummondii*, and *S. purpurea*.

3. The absorption of certain nitrogenous compounds (acetamide, urea, asparagin, and peptone) from the pitcher cavity of *Sarracenia purpurea* in the presence of a buffer phosphate solution. The buffer prevented escape of volatile nitrogenous compounds from the pitcher cavity in case they were produced prior to absorption.

4. The absorption of neutral phosphates from the pitcher cavity of *Sarracenia purpurea*.

5. The absorption of the lithium-ion from the pitcher cavity of *Sarracenia purpurea*, the tissues of which were shown spectroscopically to be free normally from the element lithium.

In each experiment with water, measurement was made of the volume of water introduced, and of the volume of the pitcher contents at the end of the experiment. In the experiments with nitrogenous compounds, either by themselves or in the presence of a buffer, and in the experiments with neutral phosphates, determination was made of the volume of solution and the mass of solute introduced, and also of the volume of the pitcher contents and the mass of solute present therein at the end of the experiment.

These studies led to the following general conclusions:

1. Water, which was introduced into the pitchers of *Darlingtonia californica* and the Sarracenias, underwent absorption.

2. When an aqueous solution of a nitrogenous compound was introduced into pitchers of the Sarracenias, both the nitrogenous compound and the water were absorbed, but at a different rate; absorption of the nitrogenous compound was usually more rapid than that of the water.

3. When a phosphate buffer was added to the aqueous solution of the nitrogenous compound, the latter was absorbed while the pitcher contents increased in volume.

4. When a neutral phosphate solution was introduced into pitchers of *Sarracenia purpurea*, both the phosphate and the water were absorbed, but at a different rate; absorption of the phosphate was less rapid than that of the water.

5. The percent of the introduced nitrogenous compound or phosphate absorbed usually increased with the period of absorption.

6. When a solution of neutral lithium citrate was introduced into pitchers of *Sarracenia purpurea*, the lithium ion was absorbed.

7. Absorption by the pitchers of substances introduced into their cavities in solution has been demonstrated (a) by the decrease in the nitrogen or phosphate content of the solution, and (b) by appearance in the pitcher tissues of lithium, an element not normally present.

8. These results indicate that the proteolytic products, formed in the pitcher cavity by digestion of the prey, are absorbed by the pitchers and are utilized for the nutrition of the plant. They also indicate that phosphates, and probably other mineral foods, derived from the prey, are absorbed and utilized in like manner.

CHEMICAL COMPOSITION OF THE TISSUES OF THE SARRACENIACEÆ

By JOSEPH SAMUEL HEPBURN, A.M., B.S. in Chem., M.S., Ph.D., and ELISABETH QUINTARD ST. JOHN, M.D.

Chemical Composition of the Dried Plants

Specimens of each North American species of the *Sarraceniaceæ* were gathered in the native habitat, in the spring or early summer unless otherwise stated. Rhizomes and pitchers of each species were collected at the same time, but were studied separately. Analyses were made of open pitchers of all species, and of closed pitchers of certain species.

The rhizomes were dug, and washed in running water until free from adherent soil. Dead tissues were removed by trimming. The rhizomes were then spread out, and dried in the air.

Closed pitchers were slit to remove the liquor, then spread out and dried in the air. Open pitchers were slit to the base of the cavity; their contents were completely removed by scraping with a blunt instrument, followed by washing in running water. They were then spread out and dried in the air. Each sample contained from 10 to 60 pitchers.

Prior to analysis, each sample was ground in a drug mill until the entire mass passed through a sieve with 20 meshes to the linear inch. The weight of the sample naturally varied with the abundance of the species and, in the case of the pitchers, with their size. The analysis was made according to the methods of the Association of Official Agricultural Chemists.[80] The results of the analyses are presented in Table XI. The water content has been reported as percent of the air-dried tissues, the other constituents as percent of the total solids. Crude fat is a synonym for ether-extract, crude fiber for cellulose. The nitrogen-free extract includes all carbohydrates except cellulose, and all organic acids. The soluble ash is that portion of the total ash which dissolved in boiling water, while the insoluble ash is the portion which was insoluble in that solvent. The alkalinity of the ash was determined by means of tenth normal hydrochloric acid, using methyl orange as an indicator; it has been reported as cc. of normal acid required to neutralize the ash (soluble or insoluble) yielded by 100 grams of total solids.

The following conclusions are based on a study of the results recorded in Table XI.

With two species (*Sarracenia Sledgei* and *S. Drummondii*), closed pitchers and open pitchers were analyzed separately. The open pitchers contained more crude fat, more crude fiber, less crude protein and less total ash than the closed pitchers of the same species.

The pitchers and the rhizomes of each species may be compared with each other. The rhizomes of *Sarracenia purpurea* were gathered at the same time and place as its "early season" pitchers. As a rule, the rhizomes of a given species contained less crude fiber, less crude protein, and more nitrogen-free extractives than the pitchers of the same species.

The insoluble ash was usually less than the soluble ash in the pitchers, while the reverse was true, as a rule, in the rhizomes.

The alkalinity of the insoluble ash was greater than the alkalinity of the soluble ash in the rhizomes of all the species and in the open pitchers of all the Sarracenias.

The nitrogen-free extractives formed more than one-half of the total solids in all but one sample of rhizomes and in all but two samples of pitchers.

The results obtained with the three samples of pitchers of *Sarracenia purpurea* indicate that the chemical composition of the plants depends, in part, on the time of the year at which they are gathered.

Total Solids and Moisture Content of the Fresh Plants

Plants of several southern species of *Sarracenia* were gathered in their native habitat in the spring, and brought to Philadelphia in the live condition. The homeopathic tinctures were then prepared from their pitchers and rhizomes. In the preparation of these tinctures, it was necessary to determine the moisture content of each specimen, by drying a 10 gram sample for 3 hours at a temperature of 100° C. The following results were obtained:—

Pitchers	*Moisture, percent.*	*Total Solids, percent.*
Sarracenia minor	65.10	34.90
Sarracenia flava	80.60	19.40
Sarracenia Drummondii	75.03	24.97
Sarracenia rubra	68.97	31.03
Rhizomes		
Sarracenia minor	58.53	41.47
Sarracenia flava	76.28	23.72
Sarracenia Drummondii	64.58	35.42
Sarracenia rubra	67.50	32.50

TABLE XI.—CHEMICAL COMPOSITION OF THE SARRACENIACEÆ.

	Genus and Species.	Weight of Sample. Grams.	Moisture.	Total Solids.							Alkalinity of Ash.	
				Crude Fat.	Crude Protein.	Crude Fiber.	Ash. Total.	Ash. Soluble.	Ash. Insoluble.	Nitrogen-free Extractives.	Soluble.	Insoluble.
Pitchers, Closed	*Sarracenia Sledgei*	12	5.48	11.61	13.22	17.95	4.15	3.08	1.07	53.07	29.85	28.55
	Sarracenia Drummondii	9.5	6.94	11.58	13.23	17.52	3.77	2.66	1.11	53.90	27.05	22.15
Pitchers, Open	*Darlingtonia californica*	60	5.14	5.04	8.04	21.33	4.36	3.70	0.66	61.23	30.70	16.15
	Sarracenia minor	33	7.56	12.13	7.84	21.96	1.87	1.17	0.70	56.20	13.85	18.60
	Sarracenia Sledgei	24	9.51	13.81	10.50	19.69	2.56	1.71	0.85	53.44	19.00	19.55
	Sarracenia flava	40	7.71	10.38	8.00	30.15	2.13	1.56	0.57	49.34	16.15	20.15
	Sarracenia Drummondii	30	9.09	14.01	10.45	24.78	3.28	2.03	1.25	47.48	19.15	27.85
	Sarracenia rubra	8	5.61	7.80	8.61	23.41	2.03	0.91	1.12	58.15	11.85	13.75
	Sarracenia purpurea early season.	42	7.15	11.72	5.47	20.98	1.91	1.18	0.73	59.92	11.75	19.50
	Sarracenia purpurea end of season.	62	6.44	6.49	7.62	17.26	2.30	1.36	0.94	66.33	6.75	22.35
	Sarracenia purpurea pitchers of preceding year, gathered in spring.	12	9.44	14.35	1.33	17.26	2.37	1.26	1.11	64.69	8.05	30.50
	Sarracenia psittacina	5	5.81	16.87	7.30	18.27	3.12	2.21	0.91	54.44	2.85	18.80
Rhizomes	*Darlingtonia californica*	8	9.83	25.04	7.91	15.57	3.06	1.60	1.46	48.42	14.30	51.00
	Sarracenia minor	35	7.30	21.08	4.72	12.67	2.61	0.28	2.33	58.92	3.45	21.15
	Sarracenia Sledgei	43	7.60	6.63	8.53	15.40	1.75	0.42	1.33	67.69	3.55	21.85
	Sarracenia flava	46	9.46	15.93	8.49	19.65	2.32	0.98	1.34	53.61	8.30	31.90
	Sarracenia Drummondii	57	7.59	10.32	5.86	13.96	3.90	2.30	1.60	65.96	7.15	27.60
	Sarracenia rubra	14	6.20	5.88	5.42	13.54	9.28	0.25	9.03	65.88	3.00	34.00
	Sarracenia purpurea	18	9.02	9.68	3.81	18.10	2.40	0.96	1.44	66.01	4.95	25.95
	Sarracenia psittacina	5	6.28	11.49	6.49	19.71	5.45	0.35	5.10	56.86	4.35	29.75

Moisture is reported as percent of the air-dried tissues, the other constituents as percent of the totals solids, alkalinity of the ash as cc. of normal hydrochloric acid required by the ash in 100 grams of total solids.

In all four species, the pitchers contained more moisture and less total solids than the rhizomes. The tissues of *Sarracenia flava* contained considerably more moisture than those of any of the other three species studied; this was true of both the pitchers and the rhizomes.

Test for Protease in the Rhizome of Sarracenia purpurea

Rhizomes of *Sarracenia purpurea* were gathered at Whitings and Davenport, New Jersey, at the end of May. Separate portions of the cleansed, crushed, freshly gathered rhizomes were extracted for 21 days at room temperature with the following solvents:—

1. Distilled water, containing 0.2 percent trikresol.
2. Hydrochloric acid 0.2 percent solution, containing 0.2 percent trikresol.
3. Sodium carbonate 0.5 percent solution, containing 0.2 percent trikresol.
4. Alcohol 50 percent by volume.

The water, the dilute acid, and the dilute alkali were used in the ratio of 10 cc. of solvent for each gram of crushed rhizome. The 50 percent alcohol was used in the ratio of 4 cc. of solvent for each gram of crushed rhizome this solvent was used since it contained approximately the same percent of alcohol as whisky, the official whisky, *Spiritus Frumenti* of the United States Pharmacopoeia[81] containing not less than 47 percent and not more than 53 percent by volume of ethyl alcohol. Whisky is frequently used as the menstruum for the preparation of household remedies from the Sarracenias.

The extracts were filtered through filter paper. The aqueous extract was a golden yellow; the insoluble residue was black. The 0.2 percent hydrochloric acid yielded a straw yellow extract, and a residue with the color of reddish brown clay. The 0.5 percent sodium carbonate gave a deep brown extract, and a deep purplish black residue. The presence of trikresol, which was used as a bactericide, possibly influenced the colors obtained with these solvents. The alcoholic extract was a reddish brown, the insoluble residue a light brown.

A series of 6 experiments was now made to test for the presence of a protease in the various extracts. In each experiment, 0.2 gram of carmine fibrin was used as the substrate. The period of incubation was 42 days at room temperature. The volume of extract used in each experiment was:—

Experiment 1. Aqueous extract 50 cc.; sufficient sodium carbonate to produce a concentration of 0.5 percent of that salt.

Experiment 2. Aqueous extract 50 cc.; sufficient hydrochloric acid to produce a concentration of 0.2 percent of that acid.

Experiment 3. Extract in 0.5 percent sodium carbonate solution 50 cc.

Experiment 4. Extract in 0.2 percent hydrochloric acid solution 50 cc.

Experiment 5. Alcoholic extract 20 cc.; sufficient sodium carbonate to produce a concentration of 0.5 percent of that salt.

Experiment 6. Alcoholic extract 20 cc.; sufficient hydrochloric acid to produce a concentration of 0.2 percent of that acid.

The trikresol functioned as a bactericide in the first four experiments, while the alcohol acted as a bactericide in the last two experiments.

Absolutely no digestion of the carmine fibrin occurred in any of the experiments. Therefore, a protease was not present in the rhizomes.

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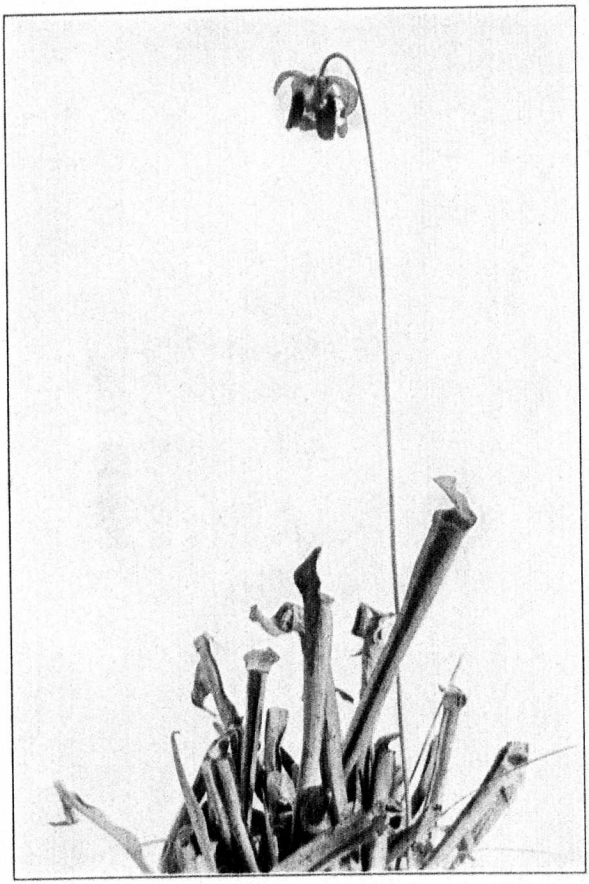
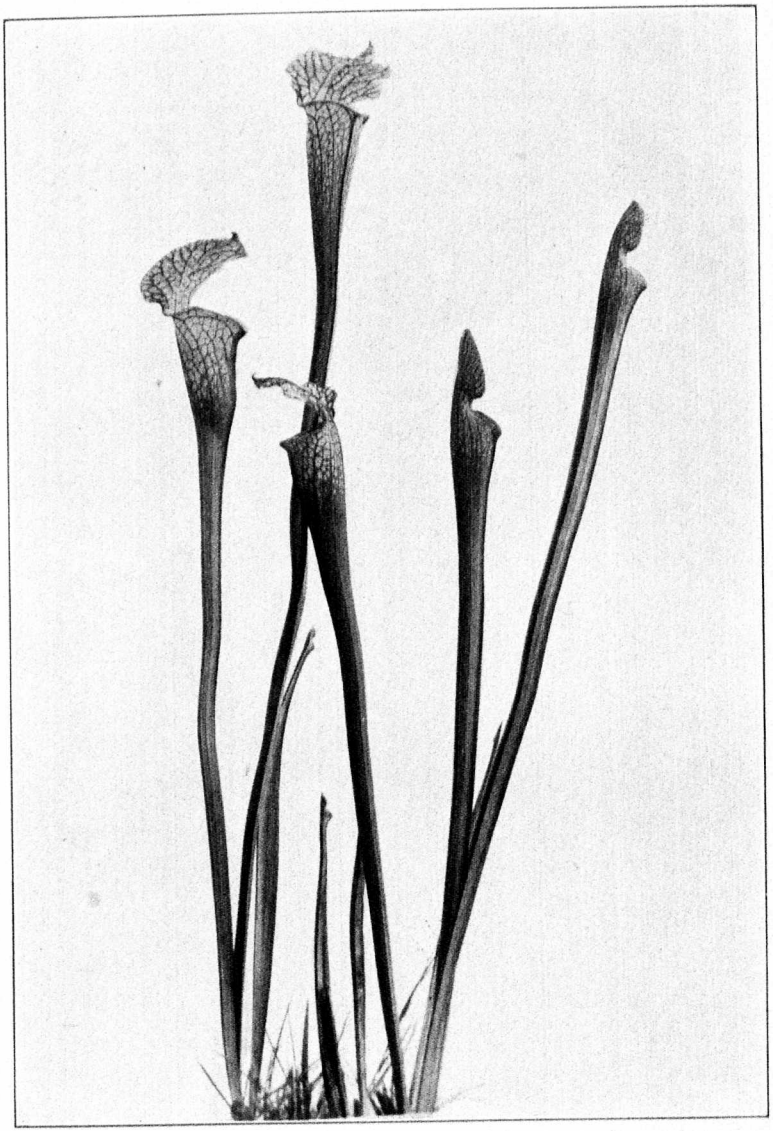
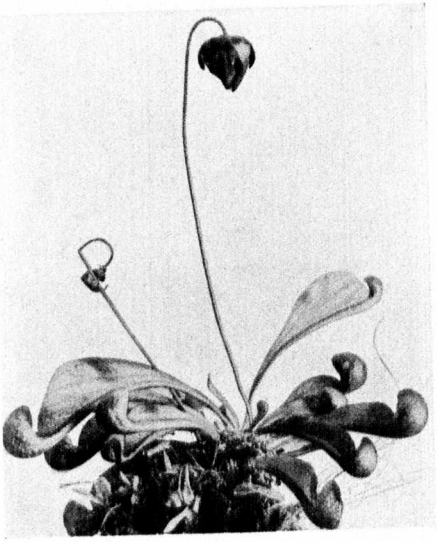


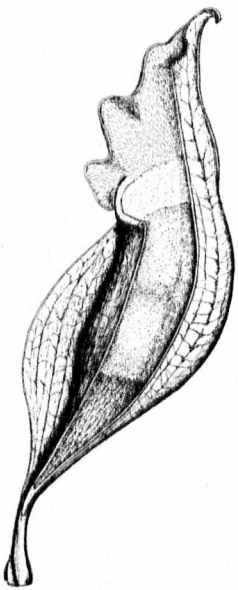
Sarracenia rubra Walt., showing clustered growth and the flower. Pitchers exceeding 60 cm. are not infrequent, but smaller forms, of from 25 to 40 cm., are more common. Photographed at Southern Pines, N. C.



S. drummondii Croom, showing fully opened pitchers and younger forms with closed lids and lips. Mobile Co., Ala., where the mature pitchers attain a height of 45 to 70 cm.

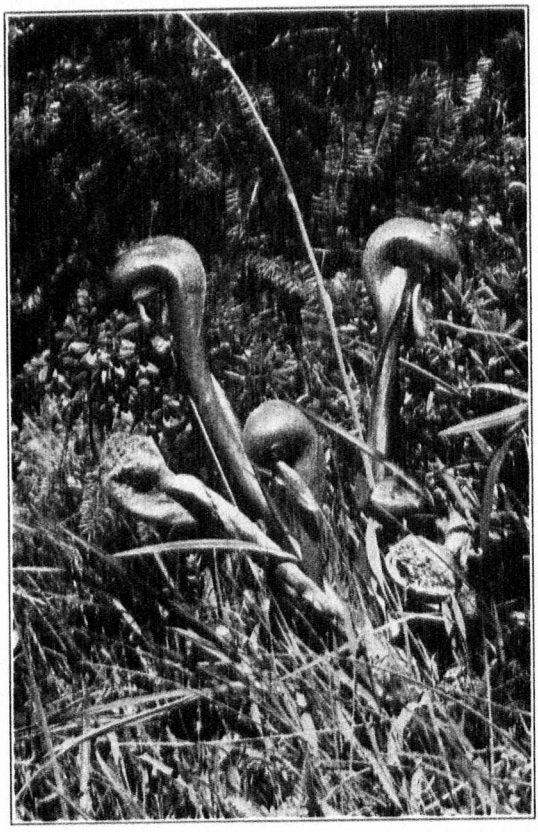


Sarracenia psittacina Michx., showing the blossom and the semi-recumbent pitchers. Photographed in southern Mississippi, where the pitchers commonly attain a length of 20 cm.

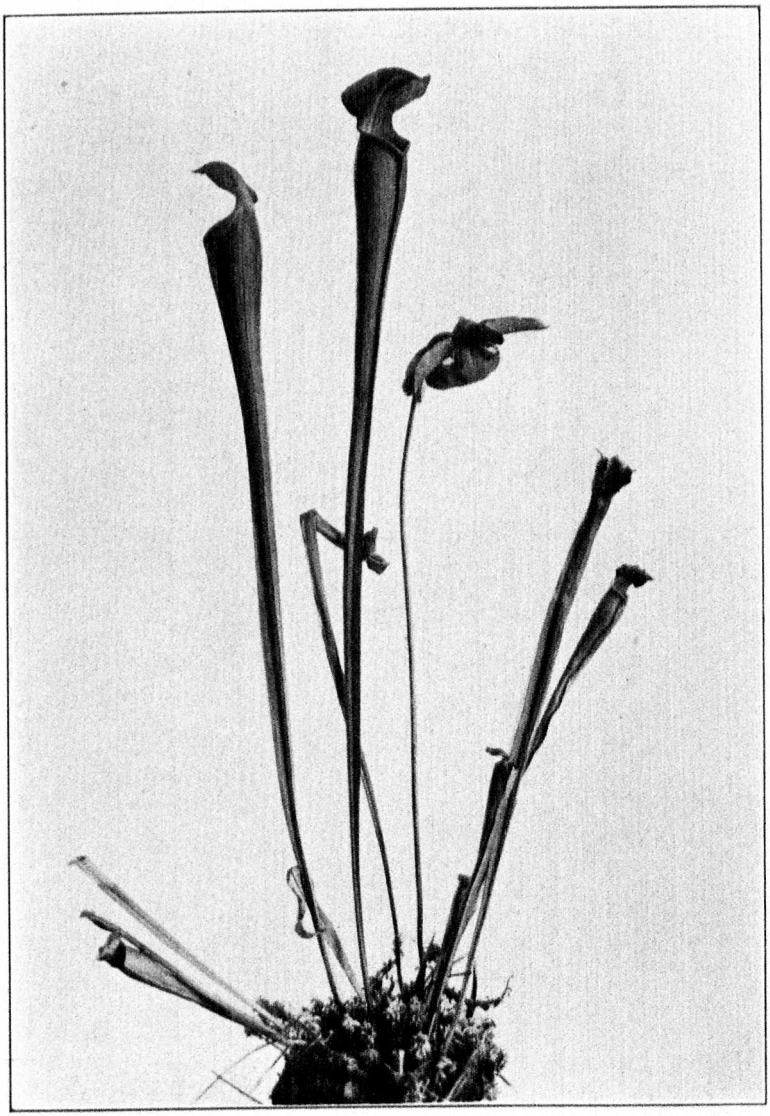


Section of open pitcher, *S. purpurea* L., according to Macfarlane, showing wing to the left, the hairy lip, the smooth conducting surface below the rim, the smooth glandular area (a species characteristic) and the detentive zone with its downwardly pointed hairs.

Sarracenia minor Walt. (*S. variolaris* Michx.), showing the hooded pitcher with its translucent windows and its yellow-petalled flower. Photographed at Summerville, South Carolina, where the pitchers of this abundant species commonly reach a height of 30 cm.

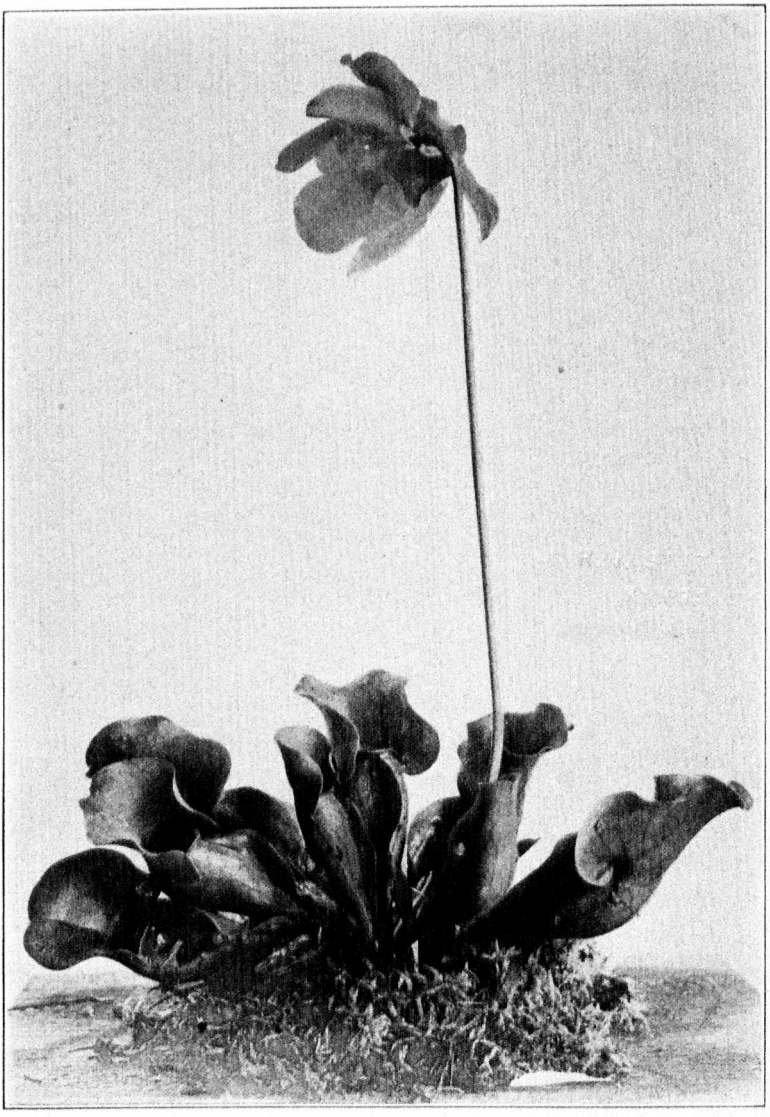


Mature plant of *Darlingtonia californica* Torr., showing twisted pitcher and expanded hood, the translucent, window-like spots, and the pendant bilobed flap. Photographed in Plumas Co., Cal., where the pitchers commonly attain a height of 50 to 75 cm.

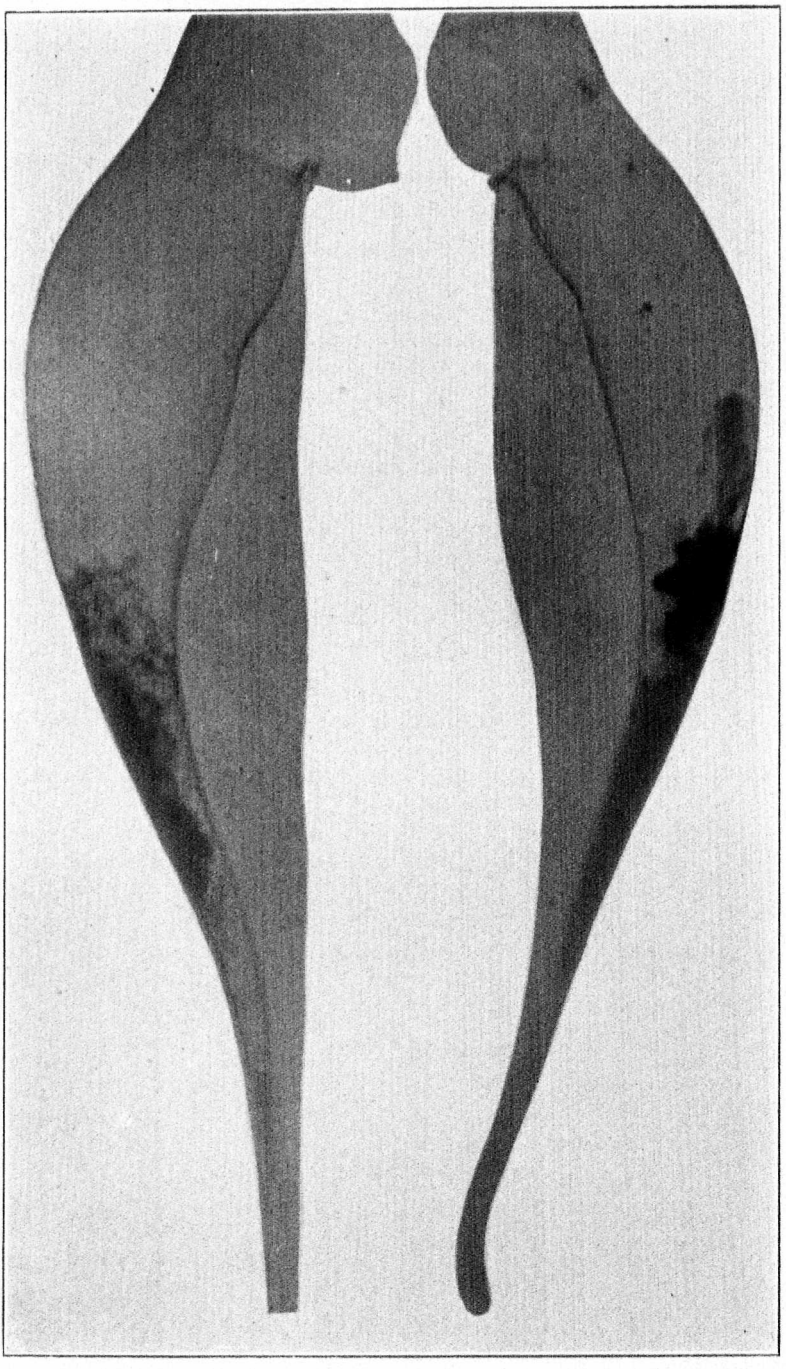


S. Sledgei Macf., showing recently opened pitchers, the flower, from which the petals have fallen, and the shriveled pitchers of the previous year. Biloxi, Miss., where the pitchers attain a height of 40 to 60 cm.

Group of *S. Flava* L. showing open pitchers. Midsummer at Southern Pines, N. C. The pitchers attain a maximum height of 100 cm., though the usual height is 50 to 75 cm.



S. purpurea L., in full bloom with open pitchers. Ocean County, N. J , where vigorous pitchers often attain a length of 25 or more centimeters



Radiograph of the pitchers of *S. purpurea* L. (full size). The liquid contents have been removed. The mass of captured insects is shown by the shadow. Moths, beetles and flies were identified. Radiograph by Goodspeed.

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